

Development of a Viscosity Measurement, Endoglucanase Assay, and Fed-batch Feeding
Apparatus for Cellulase Production Research

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Abstract

Fermentation Broth Rheology, Assay for Cellulase Enzyme Production, and Continuous Batch Substrate Addition. Ronald D. Klobberdanz (Colorado State University, Fort Collins, CO, USA 80521) K. Kadam (National Renewable Energy Laboratory, Alternative Fuels User Facility, Golden, CO 80401-3393).

Due to shortages and reliance on foreign sources of petroleum, and environmental concerns from the burning of fossil fuels, the National Renewable Energy Laboratory is researching production of ethanol from lignocellulosic biomass material. One aspect of this research is production of enzymes that are used to hydrolyze the cellulose into glucose for subsequent fermentation to ethanol. The goals of this project was to develop new techniques that will enhance enzyme production research. A new assay was implemented to measure one of the major enzyme components (endo-1,4- β -glucanase). Fermentation viscosity was also measured and modeled to gain further knowledge on enzyme broth rheology. This is important for design and scale-up of production systems. Another project was the installation of a fed-batch feeding apparatus on a 15-L fermentor. Fed-batching decreases the need to overload substrate at the fermentation start, and may improve some of the rheological problems. This system was tested and shows promise for future application.

Research Category (please circle)

ERULF: Physics Chemistry Biology Engineering Computer Science Other _____
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INTRODUCTION

Objectives

Worldwide demand for energy has been increasing for decades, and may soon surpass supply. This, coupled with the known hazardous effects of burning fossil fuels, has created an ever-increasing demand for fuel alternatives which are cheaper and burn cleaner. Ethanol is a clean burning fuel. The current dilemma is producing it in mass quantities at a price competitive with current petroleum prices. Bioethanol produced from abundant and renewable biomass resources may be the solution.

The National Renewable Energy Laboratory is engaged in research into the production of ethanol from lignocellulosic biomass. Cellulase enzyme production is one element of the overall process being developed. Cellulase enzymes are used early in the process to hydrolyze the cellulose and hemicellulose into glucose and pentose sugars (mostly xylose), which can then be fermented to the desired ethanol. The purpose of this work was to assist the cellulase production research by: (1) implementing an assay to measure endoglucanase activity, (2) determination of rheological properties of the production broth, and (3) testing and adaptation of an apparatus for fed-batch substrate addition.

Endoglucanase Assay

Cellulase enzymes are an important aspect of biomass-to-ethanol production technologies. Using cellulase enzymes to break down cellulose chains into the hexose sugar glucose creates fewer byproducts as compared to dilute or concentrated acid hydrolysis, but operates much more slowly. The complex elements of enzyme production and utilization must be better understood to allow development of more efficient cellulose hydrolysis processes. This will allow economical biomass-to-ethanol production at large scale.

One of the most important aspects of the enzyme production is understanding how the system of cellulase enzymes work together to accomplish the breakdown of cellulose. This is further complicated because different types of organisms utilize cellulose and each typically produces a unique mixture of cellulase components (Wyman, C. E., ed., 1996). Much research and development has gone into the fungus *Trichoderma reesei*, which produces a complete cellulase complex that effectively converts cellulose to glucose.

T. reesei has three separate enzymes which work concurrently on hydrolyzing the cellulose substrate. Exoglucanase cleaves cellobiose fragments from the cellulose chain. Endoglucanase acts on the internal linkages to shorten the chain fragments and produces reducing ends for exoglucanase attack. β -glucosidase converts the cellobiose to glucose. The purpose of the work was to implement an assay to measure endoglucanase (endo-1,4- β -glucanase) activity, and is based upon a previously developed procedure (Turunen, M., 1999). This assay complements the filter paper assay, which utilizes a solid substrate (Whatman #1 filter paper) and evaluates the overall activity of the three-enzyme cellulase system.

Fermentation Rheology

Rheological properties of cellulase production fermentation broth changes throughout the fermentation process as cell mass increases and insoluble solid substrate is utilized. The mycelial nature of the fungal *T. reesei* cells creates a broth which is highly non-Newtonian in nature (Ollis et al, 1995). This influences mass transfer within the reactor, limiting mixing and creating areas with higher viscosity further from the impeller. This increase in viscosity decreases the rates of transfer of mass, heat and oxygen to these areas during fermentation, and thereby limits cell growth and enzyme production. A study the fermentation broth rheology is

necessary to better understand mixing, heat and oxygen transfer requirements for this highly viscous system.

The power law and Casson models were used to describe the rheological behavior. The power law model has been shown previously to model fermentation broth and is given by

$$\tau = K (\dot{\gamma})^n \quad (1)$$

where τ (Pa) is the shear stress, $\dot{\gamma}$ (Pa·s) is the shear rate, K (Pa·sⁿ) is the power law consistency index, and n is the power law factor. One of the shortcomings of this model is its inability to account for yield stresses, which are observed in most fungal broth solutions. The Casson model has also been used to effectively model fermentation broth. This model improves upon the power law model because it takes into account the yield stress present. The Casson model is given by

$$(\tau)^{1/2} = (\tau_o)^{1/2} + K(\dot{\gamma})^{1/2} \quad (2)$$

where τ_o is the Casson yield stress (Pa) and K is the Casson consistency index (Pa·s)^{1/2}.

Fed-batch Substrate Addition

Batch operation is currently the preferred mode of operation to provide sufficient material for the fungi to hydrolyze, due to its simplicity of operation. Unfortunately, when using solid substrate, batch operation aggravates many of the rheological difficulties encountered during fermentation. One possible alternative is to operate in fed-batch or continuous mode, in which small amounts of substrate are added continuously over the duration of the fermentation life. The final portion of this research project dealt with such a system. The work encompassed receiving, setting up, and testing a solid substrate feeding system, and modifying it as necessary to accomplish reliable feeding of various substrates.

MATERIALS AND METHODS

Culture Strain *Trichoderma reesei* L27 grown on Corn Steep Liquor (CSL)-supplemented medium. A 5% (w/v) Solka-FlocTM (a purified commercial cellulase) was used to produce cellulase broth.

Equipment A Thomas-Stormer 9730-F10 Series rotational viscometer was used for all viscosity measurements. A schematic of the viscometer is provided in Appendix A as Figure 1. Cellulase production was conducted in a 15-L Braun Biostat ED15 fermentor equipped with a Digital Control Unit.

The fed-batch addition system was designed around a Model 500 Pocketball Valve and controller from Pocketball Valve Company.

Procedures

Endoglucanase Assay

Initial assays were performed on samples from a previous fermentation on which filter paper assay data had already been obtained. After the assay procedure had been finalized, the assay was also performed on samples from the rheology fermentation experiment in the 15-L fermentor.

The assay involves diluting the sample broth in deionized water to achieve a desired dilution. This diluted sample is then added to a set amount of substrate, and incubated at 50°C for 10 minutes. The reaction is then quenched using dinitrosalicylic acid, and then the samples are placed in boiling water for exactly 5 minutes to effect the required color change. The samples are then diluted again so that the sample absorbance is in the linear range of the spectrophotometer, and the sample absorbance is determined at 540 nm. This is then translated

to an endoglucanase activity based upon the sample dilution. (See Appendix B for a detailed description of the procedure.)

Fermentation Rheology

Tests were performed using a Thomas-Stormer 9730-F10 Series rotational viscometer. Baseline weight versus shear rate correlations were determined using fluids of known viscosity (i.e. varying mass percent sucrose solutions).

Solka-Floc™ was added to water to provide several slurry concentrations between 1% to 7% by weight. Other samples were generated by adding Solka-Floc™ to a 3.3% total solids (w/w) *T. reesei* broth to create slurries from 1% to 6% by weight Solka-Floc™, with total volumes of approximately 120 mL. The viscosity of these samples was then measured according to the procedure in Appendix C. The Stormer viscometer provides data in the form of a time required to turn the rotor through a specified number of revolutions, for a given weight driving the rotor. This data is converted to revolutions per second, which is then used in conjunction with the driving weight to determine a shear rate. Several runs were completed on each sample using different weights to provide curves for each slurry concentration. Sucrose solutions of varying concentration were also analyzed to provide calibration standards of known viscosity. This allows estimation of a conversion factor which relates the weight to the shear stress. With this conversion factor, the relative viscosity of the unknown can be determined.

Periodically, during the fermentation, samples of approximately 150 mL were taken and their viscosities measured. The data were correlated to the known solutions to determine a relative viscosity for the broth throughout the cellulase production process.

Samples were taken at closer intervals during the middle of the fermentation process, because this is when the enzyme production is at its highest rate, therefore, broth viscosity is also highest.

Fed-batch Substrate Addition

This portion of the work was fairly straightforward. We received the system manufactured for our purpose, set it up on a testing stand, and experimented with different operational cycles as well as different substrates. The purpose at this point was to determine the optimal operating conditions at atmospheric pressure for the different substrates used and to obtain average feed rate data. Modifications also were made to allow acceptable operation with the substrates used.

RESULTS

Endoglucanase Assay

Table 1 below shows some of the data obtained from the experiments used for the implementation. Further data is located in Appendix D.

Table 1: Representation of Endoglucanase activity for Several Fermentations

Experiment 60			Experiment 61		
Fermentor	Time (hr)	Activity	Fermentor	Time (hr)	Activity
1	168	0.496	1	168	0.433
2	168	0.552	2	96	0.378
3	168	0.399	2	120	0.371
4	168	0.428	3	96	0.165
			3	120	0.168

Fermentation Rheology

The initial set of experiments was conducted using the power law and Casson models to estimate the apparent broth viscosity relative to known viscosity standard solutions.

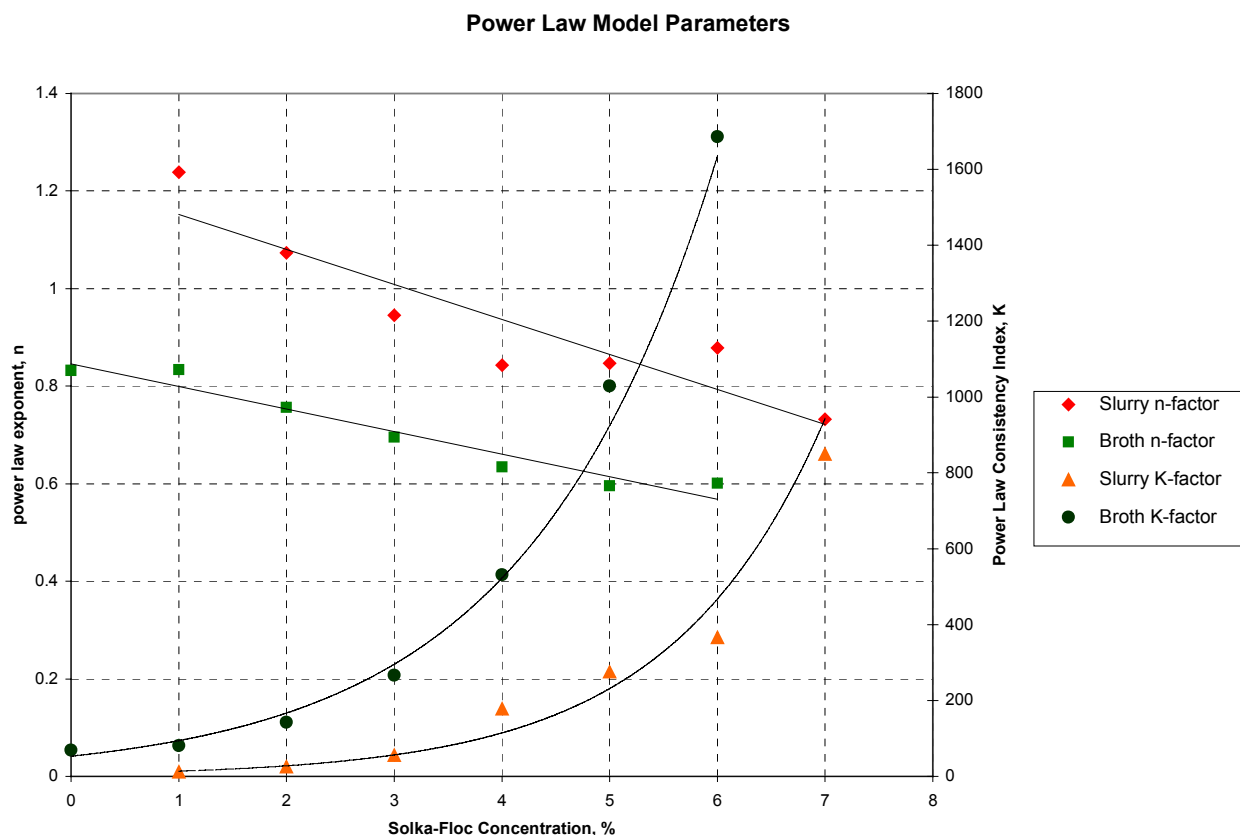


Figure 2: Representation of Power Law Model Parameters for Model Construction

For the power law model (Figure 2), the power law exponent, n , slowly decreases from about 1.0 in a nearly linear fashion as the Solka-FlocTM concentration increases. This is expected as the slurry becomes more viscous, the power law exponent should decrease (i.e., becoming pseudoplastic). The power law consistency index, K , increases as the solution concentration increases, in an exponential manner, due to the non-Newtonian nature of the slurry. The index is significantly different when comparing the pure Solka-FlocTM slurries to the Solka-FlocTM with broth. This illustrates the significant effect the fungal mycelia have on broth rheology.

The Casson model yield stress for mycelial broth, τ_o , as noted in Figure 3, increases in an exponential manner, starting near zero at low Solka-FlocTM concentrations, but increasing rapidly to near 3000 mPa. This makes sense for these solutions, because they have demonstrated

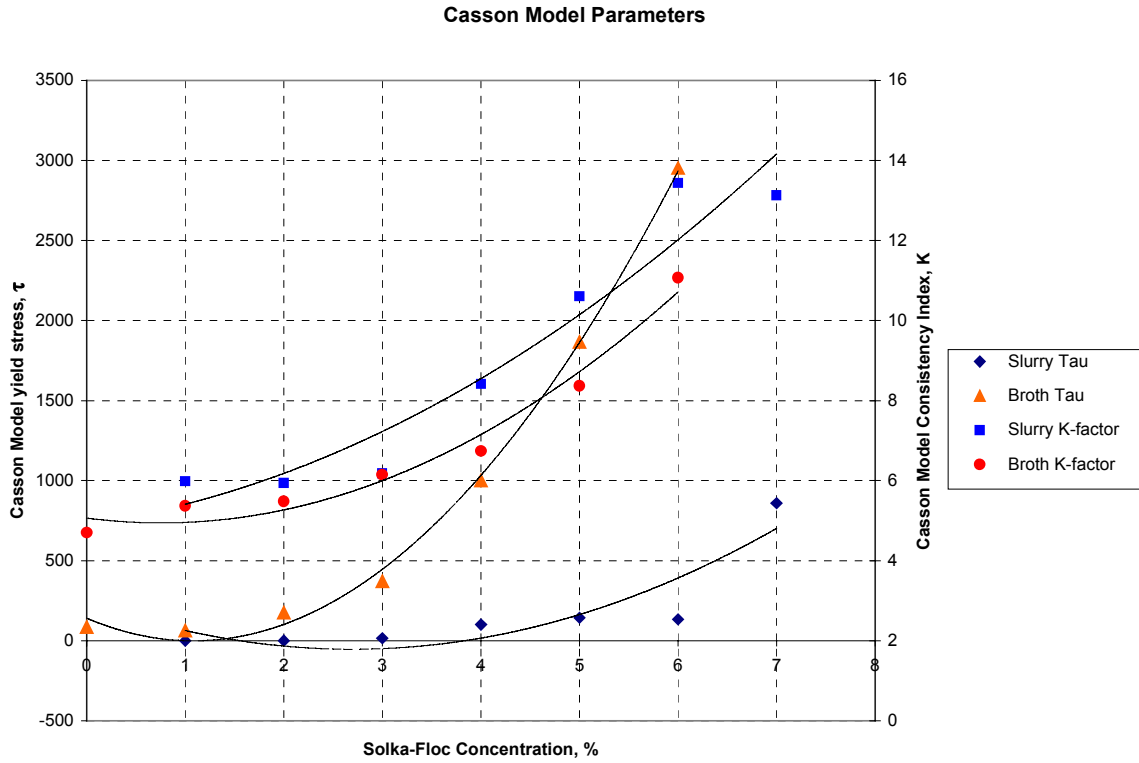


Figure 3: Casson Law Model Parameters for Model Construction

previously a yield stress which must be surpassed before motion will occur (Ollis et al, 1996; Karanth et al, 1982) . The Solka-Floc™ solutions exhibit a slight yield stress, which remains around 100mPa until the solution reaches 7% by weight, when it increases dramatically to 860mPa. This unexpectedly high yield stress is as yet unaccounted for. Ignoring this reading, an average yield stress of below 100mPa is obtained for pure Solka-Floc™ slurries. These findings show that fungal mass creates a substantial yield stress, which is magnified by the addition of particulate substrate.

The results of the fermentation rheology measurements are interesting, but further experimentation is needed to corroborate the data. There were difficulties encountered during the sample collection that may have affected the data quality. Due to the high solids content of

the solution early in the fermentation, for example, it was difficult to collect samples. And, after the first samples were collected, a leak developed from the collection valve, which continued for a good portion of the fermentation and possibly affecting subsequent samples.

The production broth samples were compared with model samples which should be representative of the broth at the sample time. The model for pure 5% Solka-Floc™ slurry was used for assessment of the apparent viscosity of the initial fermentation slurry, because that solution most resembles the composition of the broth at startup. As shown in Table 2, the power law model correlates, but the Casson model does not.

Table 2: Fermentation Rheology Comparisons with Models

Starting Condition Comparison			Mid-fermentation Comparison			Near End Fermentation Comparison			
	5% (w/v) SF	t=0 Broth		2% SF & Broth	t=23 Broth		3% (w/v) SF	t=102 Broth	1%SF & Broth
Power Law			Power Law			Power Law			
n=	0.847	0.642	n=	0.756	0.725	n=	0.945	0.874	0.834
K=	276	272	K=	143	126	K=	56	63	82
Casson			Casson			Casson			
τ=	144.0	10.6	τ=	175.0	176.0	τ=	15.3	28.1	64.1
K=	480.0	5.0	K=	5.5	4.6	K=	6.2	5.5	5.4

For a mid-fermentation comparison, the 23 hour sample broth was compared to the 2% substrate in broth model. Both the power law and Casson models appear to be reasonably predictive at this point. Near the end of the fermentation (time=102 hours), a 1% Solka-Floc™ in broth model is expected to mimic the findings, but only the power law model appears reasonably predictive. The power law exponent is slightly higher, whereas for the Casson model, the Casson yield stress is much lower than expected.

The power law can be stated as: $\tau = K (\dot{\gamma})^n$

This can be expanded to yield: $\log \tau = \log K_p + n \log \dot{\gamma}$

This form can then be compared to the Casson model: $(\tau)^{1/2} = (\tau_o)^{1/2} + K(\dot{\gamma})^{1/2}$ to explain the similarities in the behavior between $(K_p \text{ and } \tau_o)$, and $(n \text{ and } K_c)$, as shown in the figures in Appendix E.

Fed-batch Substrate Addition

Results for the initial tests of the Pocketball batch addition system were promising. As Table 3 below and Figure shows, all utilized substrates flowed well. Due to the small amounts of corn stover available for these tests, the test runs for the dry stover were performed over only a fraction of the number of cycles (7 to 15) as were completed on the other samples (50 cycles). The difference between the three corn stover samples used was their moisture content (Table 2 in Appendix F). A second run was performed with sawdust due to an unusually high flow rate during several cycles in the first run.

Table 3: Averages and Standard Deviations of Flow for Different Solid Substrates (grams)

	SF (g)	Sawdust	Sawdust #2	Wet Stover	1 week Dry Stover	4 month Dry Stover
Avg (g)	0.15	0.80	0.60	1.00	1.91	2.67
Std Dev	0.068	0.520	0.248	0.554	0.667	0.331
Variance %	44.9	64.6	41.6	55.4	35.0	12.4

There were, of course, some obstacles to overcome. The system uses air to purge the inlet and outlet of the pocket valve to maintain good metered flow into and out of the valve. With dry Solka-Floc™ as substrate, the upper air purge was too powerful, blew substrate onto the hopper walls, and through the hopper lid. This was remedied by reducing the purge pressure and time, and sealing the hopper lid with a gasket.

The same purge also caused a dilemma with heavier substrates, such as wet corn stover or sawdust. The purge would create a pocket over the top of the valve, and the material above the pocket would create a bridge and would not allow flow into the valve. Several different ideas

were attempted, and stirring of the substrate appeared to provide the best results. Therefore, another modification was done to add an automatic stirrer powered by an electric motor to the lid of the hopper. The addition of this stirrer, in conjunction with the short air purge produced consistent and reliable feeding. Table 1 in Appendix F shows the operating cycle that was finally determined to generate acceptable results.

The next stage of the testing was under fermentation conditions on a pilot scale fermentor. Unfortunately, a controller malfunction halted testing and the unit was sent to the manufacturer for repair.

CONCLUSION

Ethanol production from renewable biomass will become an important fuel source in the near future. The production of cellulase enzymes at a sufficient level to make this endeavor economical imposes challenges. The research performed here will assist future cellulase production efforts.

The implementation of the endoglucanase activity assay will aid in the understanding of the cellulase enzyme complex. This assay will complement the existing filter paper activity assay and will be useful in assessing cellulose quality.

The research examines how the rheological properties of the production broth vary as the fermentation progressed, and developed models based upon the power law and Casson equations which describe apparent broth viscosity as a function of substrate slurry concentration. The models can be used to generate viscosity data that will be useful for design of production processes.

Both models can be used to estimate broth apparent viscosity at discrete times in the process, and corroborate previous research (Ollis et al, 1996). But, as was stated in the results, points on the model that should have coincided had a larger discrepancy than expected, and the Casson model appeared to fail at low concentrations of cell mass when compared to the models. But the results do appear to match somewhat the graphs generated by Ollis et al, although the yield stress values for our experiment were much lower. Continuing data collection is needed to better correlate the data, and to resolve possible inconsistencies caused by the compromised sampling conditions or otherwise.

Finally, a new fed-batch addition system for a 15-L fermentor was tested. Results show that this system has great potential for future application to cellulase production. Initial testing was unable to complete more than a few runs before difficulties were encountered with the substrate flow within the hopper. Addition of the stirring unit (Appendix F, Figures 2 and 3) solved the problems. Subsequent testing showed good operability. Tests utilized only short-term runs of up to 50 cycles, with the flow average and standard deviations calculated between individual cycles throughout the run. Because of this, the deviations in the data for some of the substrates appear unusually high. Over long-term operation, this deviation should not prove to be a large factor. Further testing to determine optimum operating conditions may also decrease any deviations between individual cycles. As shown in the two runs using sawdust, there is some variance between runs even with no changes in operating conditions. Unfortunately, minor setbacks delayed testing and final tests with the system in place on the fermentor could not be accomplished prior to my departure.

ACKNOWLEDGEMENTS

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APPENDIX A

Figures

- 1 – Thomas Stormer Viscometer**
- 2 – Power law model parameters**
- 3 – Casson law model parameters**

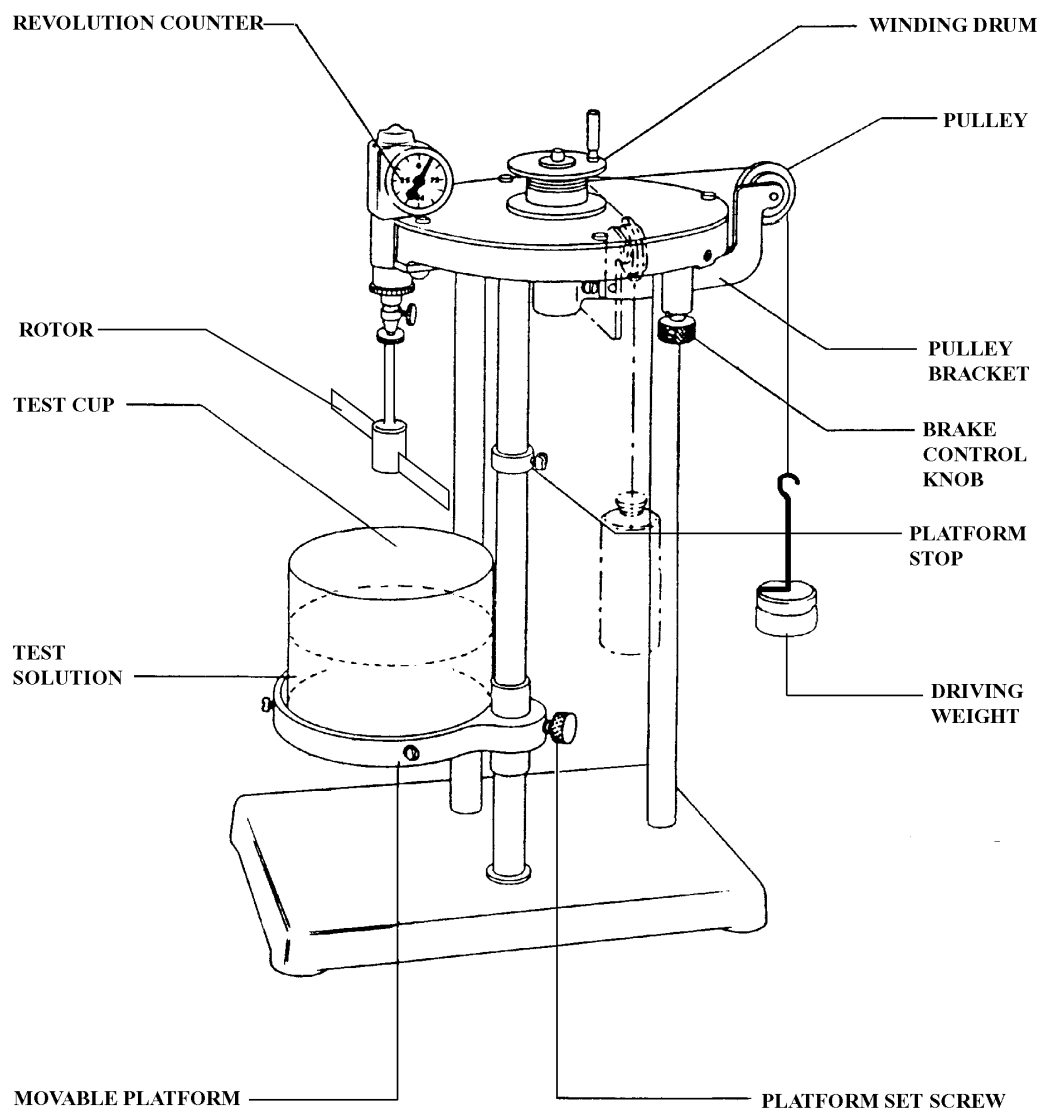


Figure 1
Schematic of the Thomas®-Stormer® rotational viscometer

Power Law Model Parameters

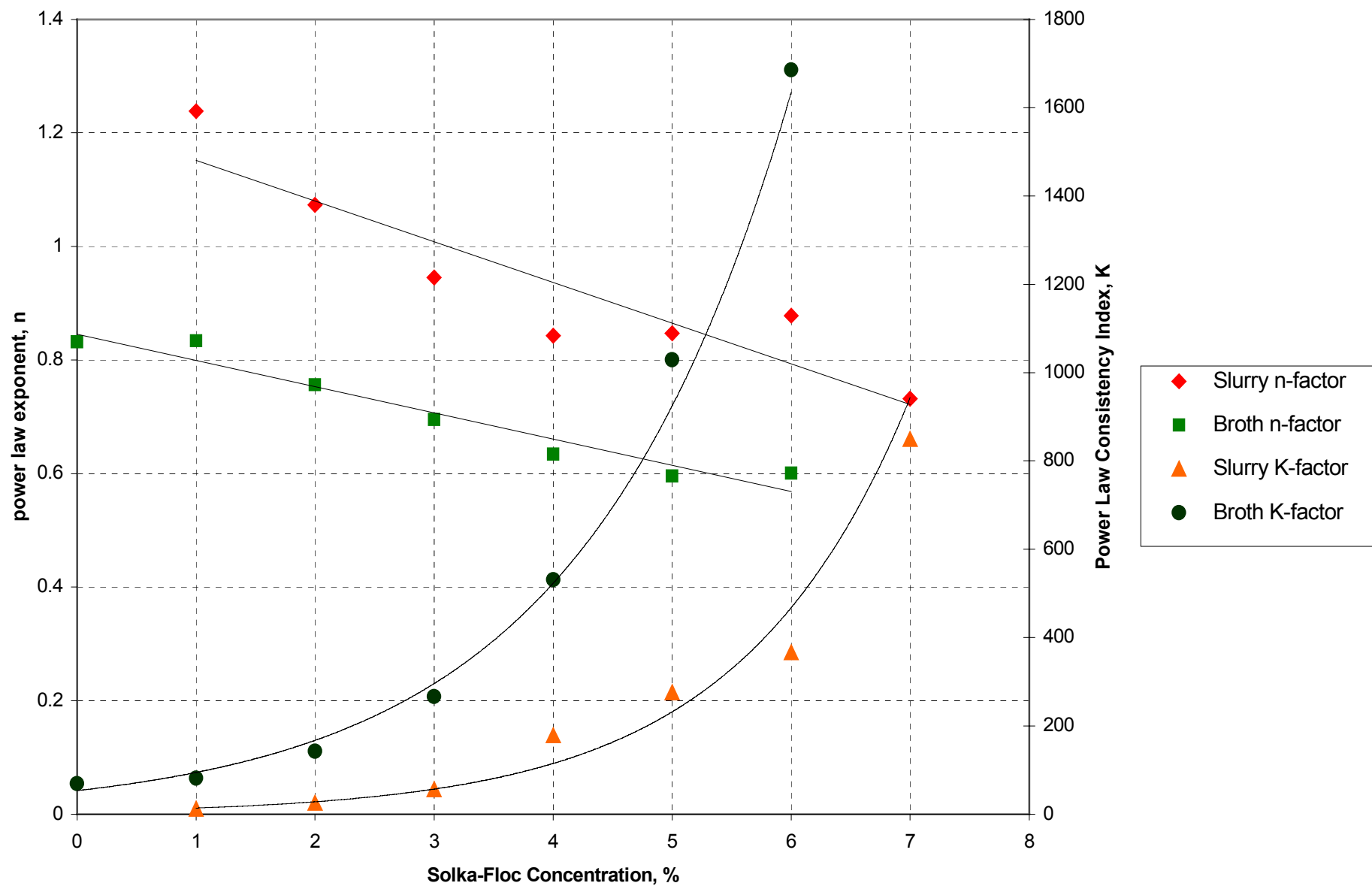


Figure 2: Estimation of Unknown Parameters for Power Law Model

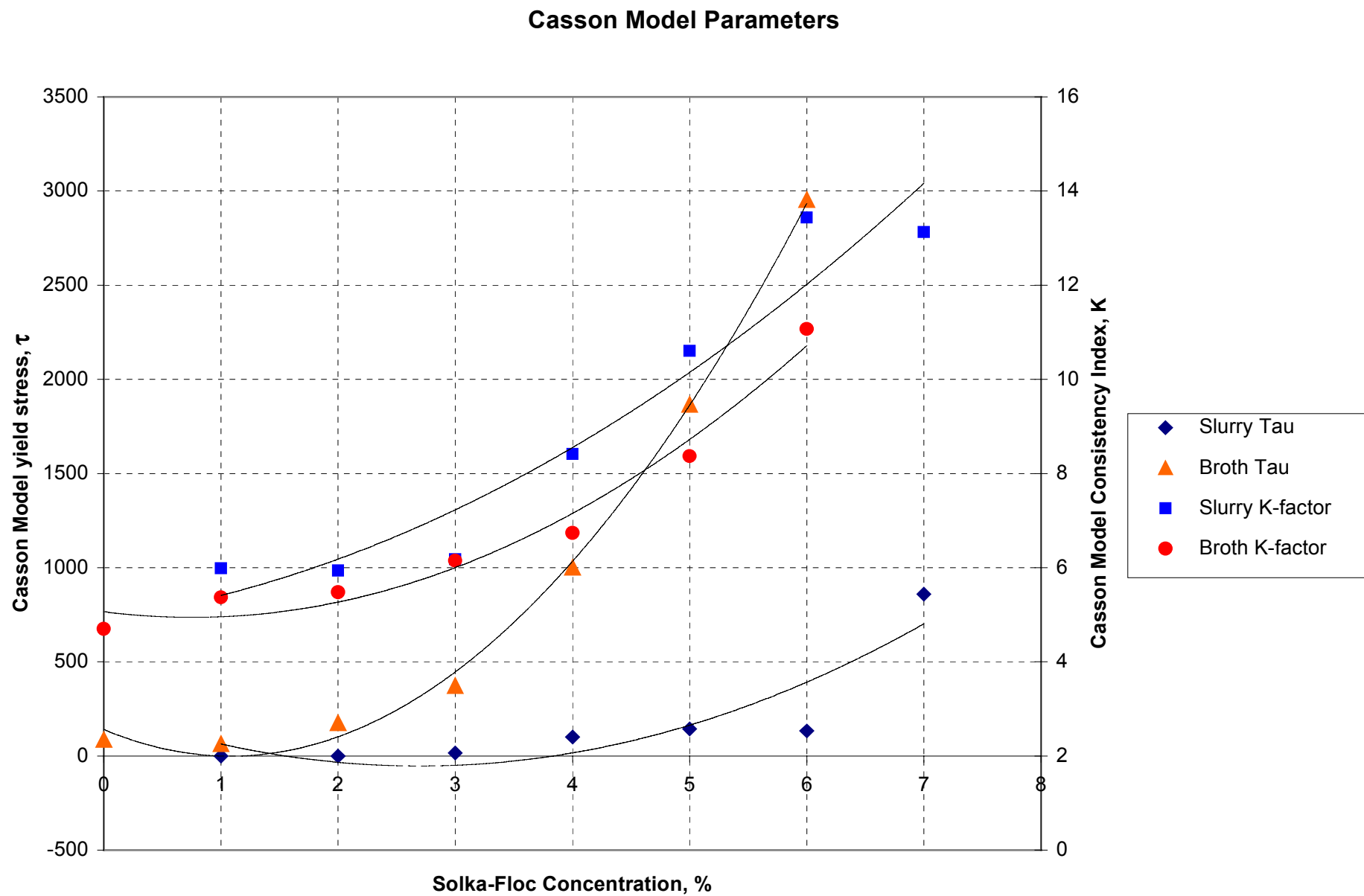


Figure 3: Estimation of Unknown Parameters for Casson Model

APPENDIX B

Procedure for the assay of endo-1,4- β -glucanase enzyme

Procedure: Assay of Endo-1,4- β -glucanase Activity

Principle:

Endo-1,4- β -glucanase in the sample hydrolyzes the substrate, hydroxyethyl cellulose, and the reducing sugars produced are assayed spectrophotometrically.

Unit of Activity:

One Endo-1,4- β -glucanase unit (ECU) is defined as the amount of enzyme producing one nmol of reducing sugars as glucose in one second (1 ECU = 1 nkat).

Reagents and Equipment Needed:

1% hydroxyethylcellulose (HEC) in 0.05M sodium citrate buffer (pH = 4.8) (Substrate)
0.05M sodium citrate buffer
0.1M glucose standard
1:20 diluted CPN enzyme standard
Sample enzymes
DI water
Dinitrosalicylic acid (DNS)
Test tubes
Test tube racks
Water bath @50.0°C
Water bath @100.0°C (crock-pot)
Pipettes and tips (1000 μ and 250 μ for assay using 2 mL total volume)
Stopwatch

Procedure:

1. Remove and allow CPN standard to thaw. If not used immediately after thawing, store vials on ice.
2. Prepare samples of different dilutions
Desired absorbance range at 540 nm is 0.20 to 0.25.
Examples:

Dilution Factor	Amount of enzyme broth	Amount of DI water	Final Volume
1:10	0.100 μ L	0.900 μ L	1000 μ L=1 mL
1:15	0.067 μ L	0.933 μ L	1000 μ L=1 mL
1:20	0.050 μ L	0.950 μ L	1000 μ L=1 mL

- a) Add proper amounts of enzyme sample to DI water, based on dilution calculations.
- b) Vortex mix each sample to ensure homogeneity.
3. Create glucose standard dilutions, ranging from 1:10 to 1:100 dilutions, using 0.1M glucose solution in citrate buffer to make 2.0 mL. For example:

Dilution Factor	0.1M Glucose, mL	Sodium citrate buffer, mL
1:10	0.200	1.800
1:15	0.133	1.867
1:25	0.080	1.920
1:50	0.040	1.960
1:100	0.020	1.980

4. Set up and label sample tubes.
 - a) Add 1.8 mL substrate to each tube.
 - b) Add 0.2 mL citrate buffer to the tube to be used as a blank.
5. Place tubes in 50.0°C water bath.
 - a) Place marbles on top of all tubes.
6. Set up a second set of tubes for a dilution blank, in the same arrangement as used above for the samples.
 - a) Add 4.5 mL DI water to each tube.
7. In an incremental fashion, add 0.2 mL of the diluted enzyme samples to each sample tube (at 30 second intervals), except for the blanks and glucose standards.
 - a) Vortex mix each sample tube.
 - b) Replace in the water bath.
 - c) Replace the marble on top of each tube.
8. When the first sample has been incubating for exactly 10 minutes, add 3.0 mL DNS to quench the reaction. Repeat for all samples, using the same incremental timing method.
 - a) Replace each sample in the water bath.
 - b) Replace the marble.
9. After all samples have been quenched, place the set in the 100.0°C water bath to boil for exactly 5 minutes.
 - a) Remove the sample rack and place it in the ice bath.
10. When the samples have cooled, remove the marbles and extract 0.5 mL of each sample and add to the corresponding dilution blank tube. This dilution is necessary to place the samples within the linear range for absorbance on the spectrophotometer.
 - a) Vortex mix each tube.
11. Read the absorbance from spectrophotometer and record.
 - a) Ensure that spectrophotometer is on and warmed up.
 - b) Ensure that the correct range is set (540 nm). To set the wavelength, press 5-4-0 on keypad, then press '*Second Function*', then '*Go To λ*'

12. Zero the photometer using the dilution blank.
 - a) Insert the test tube blank. Ensure that it is straight in holder (turning the tube while inserting helps).
 - b) Press '*Second Function*', then '*Zero A*'.
 - c) Remove and reinsert the tube several times, to ensure that it is zeroed correctly.
13. Read each sample tube and the glucose standards, and record the results.
14. Re-read the dilution blank to ensure the zero is still correct.
15. Waste disposal
 - a) Waste from all tubes having DNS in them must be disposed of in the proper hazardous waste container in the Fermentation I Lab.
 - b) Test tubes are then rinsed and placed in glass waste containers.
 - c) DNS waste logsheet: Write the date, initial, and the amount of waste deposited in the waste container (estimate by counting the number of tubes to which DNS was added, then multiplying by 10).

Calculation:

Plot the standard curve using the absorbance values obtained from the glucose standards with concentration on the y-axis and absorbance at 540 nm on the x-axis. Linearly regress the data (R^2 should exceed 0.90).

Endoglucanase activity is obtained by multiplying the glucose concentration ($\mu\text{mol/mL}$) by 1000 and dividing by the time of hydrolysis, 600 seconds.

$$activity = \frac{conc \cdot 1000}{600}$$

The activity for the samples are then determined by reading from the standard curve for the corresponding absorbance and multiplying by the dilution factor.

Reagent Preparation:

- 1) Preparation of 1% HEC:
 - Dissolve 1g HEC in 0.05M sodium citrate buffer (pH=4.8) in a volumetric flask.
 - Dissolve incrementally.
 - Bring volume to 100 mL with the buffer.
 - Stir for at least one hour using magnetic stirrer, until the mixture is homogeneous and clear.
- 2) Preparation of 0.1M Glucose solution:
 - Dissolve 1.802 g glucose in 0.05M sodium citrate buffer (pH=4.8) in a volumetric flask.
 - Bring volume to 100 mL with the buffer.
 - Filter sterilize.

Precautions:

- ❖ DNS is a phenol and highly toxic; gloves and safety glasses should be worn when handling.
- ❖ CPN standards are kept in -76° Freezer.
- ❖ Incubation time is 10 minutes, therefore, performing an assay using more than 16-18 sample tubes will create a timing problem with enzyme addition conflicting with DNS quenching.
- ❖ Test tubes must be straight when placed into the holder in the spectrophotometer. The holder does not hold the tubes exactly in the same manner each time. Turning the sample tubes while inserting helps to ensure they are straight, and repeated removal and reinsertion until several consistent readings are attained will ensure accurate results.
- ❖ Marbles act as a simple reflux condenser and ensure that the sample concentrations do not change due to evaporative losses during incubation and boiling.

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APPENDIX C

Procedure for Viscosity Measurements

Background:

The Thomas[®]-Stormer[®] 9730-F10 series viscometer is a rotational shear-type viscometer, which can be used to determine the rheological properties of many different types of fluids.

Principle:

The 9730-F10 can be used to determine a fluid's rheological properties by measuring the time required to turn the rotor through a specific number of revolutions for different driving forces. The time required is then converted to a shear rate. When used with fluids of known viscosity, the weights used can be converted to a shear stress. This allows the estimation of rheological parameters of the unknown fluid.

Apparatus:

Figure 1 on page 4 shows a schematic of the viscometer.

Parts Description:

- Revolution counter – counts the number of revolutions of the rotor.
- Rotor – turns in response to the weight.
- Movable platform – for placement of the sample fluid; height adjustable.
- Platform set screw – used to hold the platform at the optimum desired test level.
- Driving weight – variable weight platform allows the application of different weights, and hence, shear stress.
- Platform stop – top stop limit for the test solution platform.
- Brake control knob – controls the movement of the rotor.
- Winding drum – used to set the revolution counter and return the weight to the starting position.

Reagents and Equipment Needed:

- Thomas[®]-Stormer[®] 9730-F10 series viscometer
- Weight set (1, 2, 5, 10, 20, 50, 100, 200, 500 g)
- Sample container
- Sample
- Timer
- Thermometer (optional – needed if viscosity measurements taken at $T \neq$ room temp.)
- Viscosity standards (from Thomas)
- Other standards for non-Newtonian fluids, e.g., Sucrose solutions.

Initial Setup:

1. Check the apparatus for proper working order and no visible problems. Ensure all parts are clean and dry.
2. Place the instrument on a flat tabletop or shelf so that the driving weight has approximately 40 inches of unobstructed distance to drop through. This allows the rotor to spin through approximately 125 revolutions.
3. Run the cord over the pulley and wind it uniformly on the drum (turn counter-clockwise).
4. If not already so, set the revolution counter to approximately 80-90. This allows 'spin-up' time for the impeller to reach full speed before timing begins.
 - a) Release the brake by pulling down and turning $\frac{1}{4}$ turn, allowing the weight pedestal to drop, and the indicator to spin to 80-90.
 - b) Reset the brake
 - c) Turn top handle counter-clockwise to rewind cord.
 - d) To adjust, release the brake and turn the handle clockwise or counter-clockwise to adjust, then reset brake.
5. Move the sample stand down fully by releasing the platform set screw.
6. Set the platform stop limit such that the impeller will be completely immersed within the sample fluid, but not touching the bottom of the container.
7. Place the sample container on the stand, and move the stand up to the platform stop, securing by tightening the platform set screw.
8. Ensure that the sample container is centered under the impeller.

Operation:

1. Prepare the timer by zeroing it.
2. Release the brake on the viscometer.
3. Begin the timer when the revolution counter passes zero.
4. Continue timing until the counter again reaches zero, then stop the timer.

Resetting the Device:

1. With the brake released, rotate the handle CCW to rewind the cord and reset the revolution counter to ~80-90.
2. When the revolution counter has reached ~80-90, reset the brake and continue winding the cord until the weight stand is at the top.
 - a) With a very viscous sample, the sample may need to be lowered until the impeller is out of the sample to allow rewinding.
 - b) The mechanism is designed to slip somewhat to avoid damage, and may do so if rewound with the impeller still in a viscous sample.
3. Repeat the above operation and resetting steps for each sample test.
4. Perform several runs at each weight to yield an average time.
5. Adjust the weight as appropriate to begin a new data point.

Cleanup:

Lower the sample stand.

Lower the stand only partially if finished with the sample, and rinse the impeller into the sample container.

Wipe impeller clean.

Ensure all portions of the instrument are clean and clear of sample.

Remove the sample and dispose of properly.

Remove weights and restack on the weight rack.

Precautions:

- Use appropriate weights to avoid splashing of potentially hazardous liquids, and to avoid damage to the instrument.
- The brake is not designed to support heavy weight. If required, heavy weights should be added after the rotor has been immersed in the sample and removed before the sample is lowered and the cord is rewound.
- Cord winding:
 - To ensure proper operation, the cord must be wound smoothly, with constant tension.
 - Avoid winding the cord over itself.
 - Ensure the cord winds properly around the spindle, and does not wind around the shaft.
- A minimum time of 20 seconds should be used to avoid turbulence that could alter readings and calculations.
- For further operational alternatives, or for maintenance instructions, see the Operating Instructions (1).

References:

1. 9730-F10 series Thomas-Stormer Viscometer Operating Instructions, Thomas Scientific Technological Service, Swedesboro, NJ, pp. 1-7.

APPENDIX D

Endoglucanase Assay Data

- 1 – Experiment 60 Glucose standard curves**
- 2 – Experiment 61 Glucose standard curves**
- 3 – Experiment 60 data**
- 4 – Experiment 61 data**

Glucose absorbance vs dilution Experiment 60 data

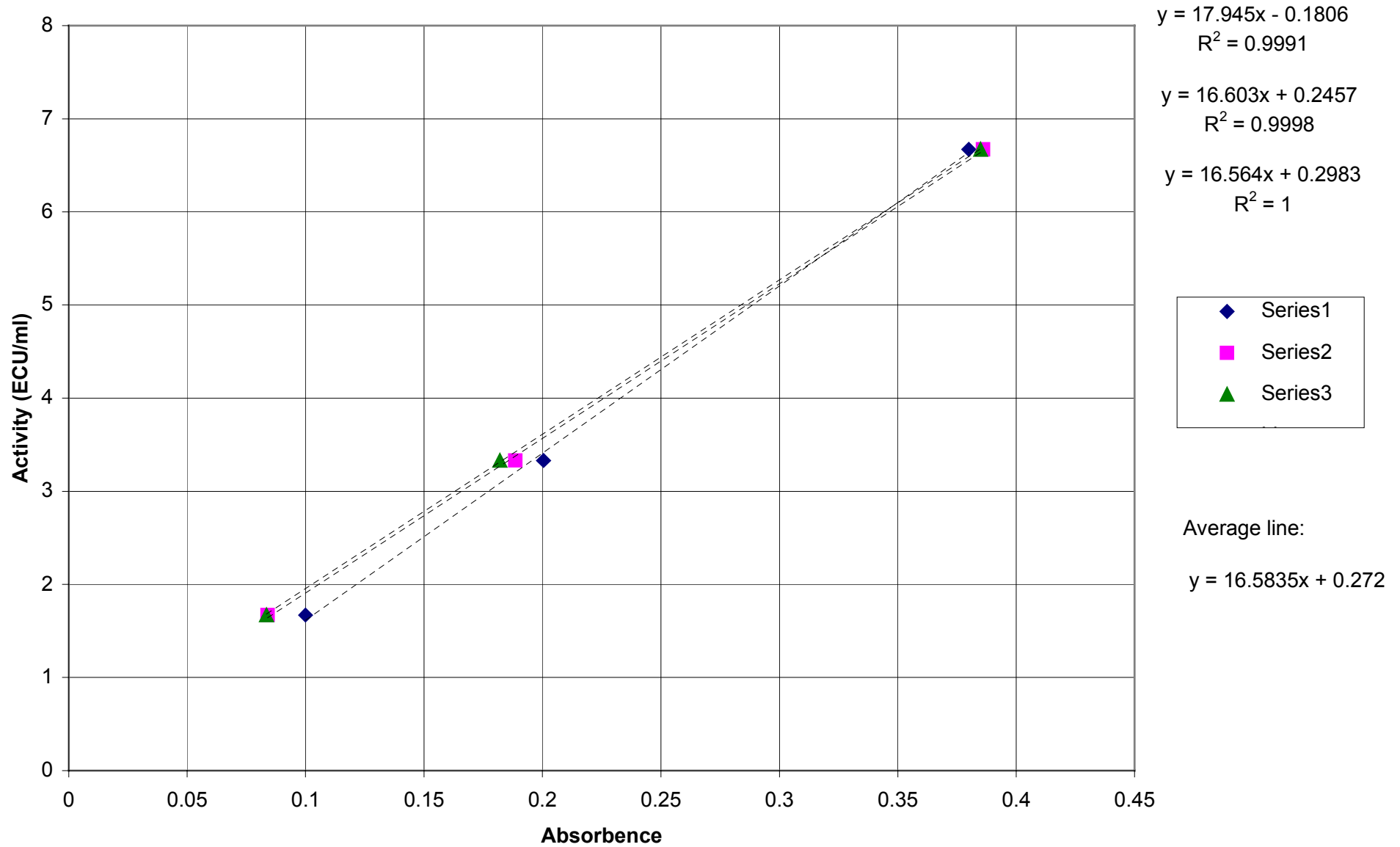


Figure 1: Glucose Standards Curves for Experiment 60 Data

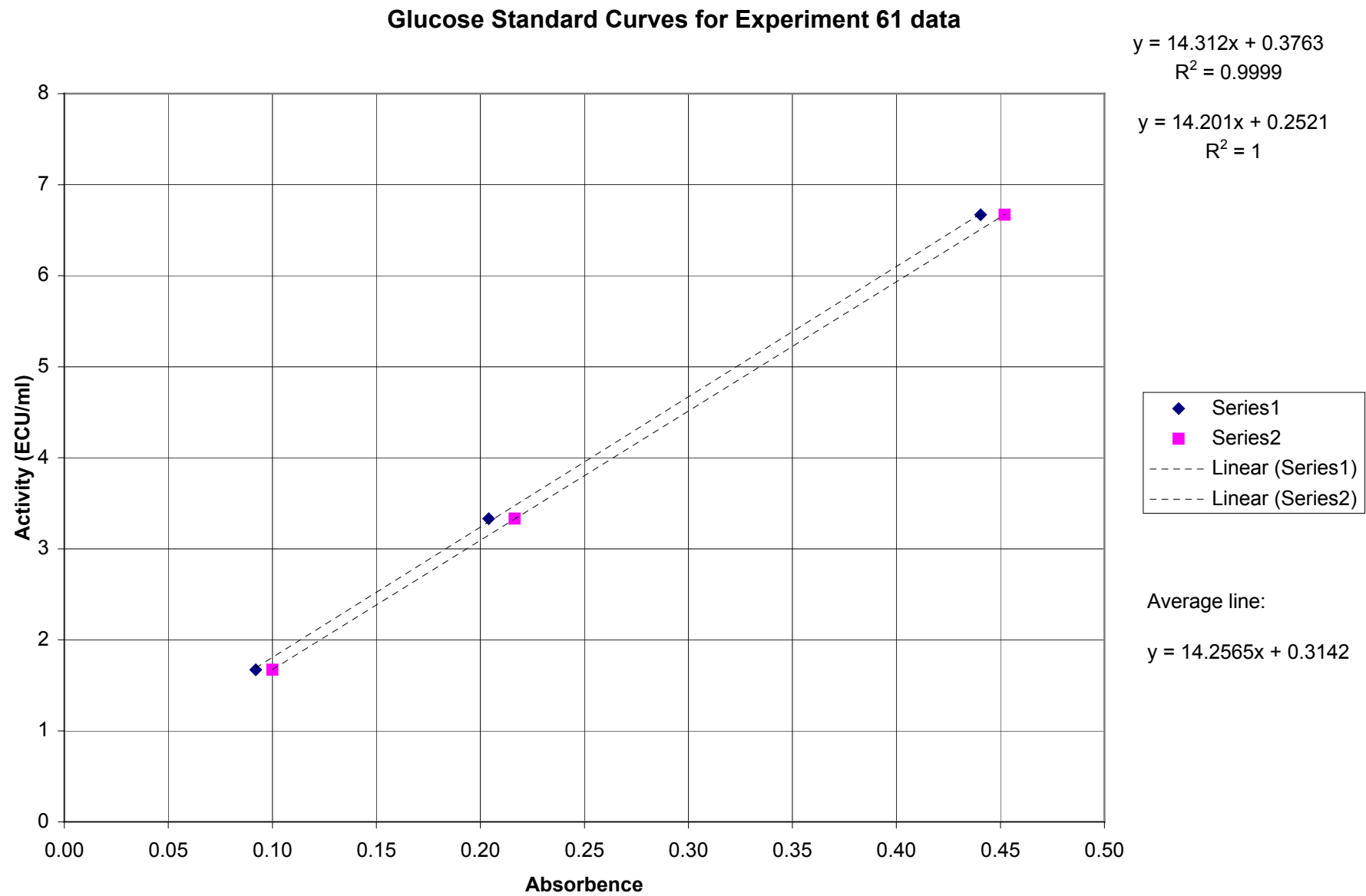


Figure 2: Glucose Standards Curves for Experiment 61

	Sample	e60f1s8		e60f2s8		e60f3s8		e60f4s8	
Run		Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
1	Abs. dilutn/avg Activity	0.180	0.180	0.220	0.222	0.202	0.217	0.240	0.243
		10	0.180	10	0.221	10	0.210	10	0.242
			0.326		0.394		0.375		0.428
	Abs. dilutn/avg Activity	0.121	0.132	0.184	0.180	0.153	0.154	0.206	0.203
		20	0.127	20	0.182	20	0.154	20	0.205
			0.118		0.165		0.141		0.183
2	Abs. dilutn/avg Activity	0.230	0.234	0.250	0.251	0.232	0.241	0.269	0.275
		7	0.232	10	0.251	10	0.237	10	0.272
			0.588		0.443		0.419		0.478
	Abs. dilutn/avg Activity	0.229	0.229						
		8	0.229		0.000		0.000		0.000
			0.509		#DIV/0!		#DIV/0!		#DIV/0!
3	Abs. dilutn/avg Activity	0.217	0.216	0.251	0.249	0.226	0.227	0.274	0.270
		8	0.217	8	0.250	10	0.227	7	0.272
			0.483		0.552		0.403		0.683
	Abs. dilutn/avg Activity							0.265	0.262
			0.000		0.000		0.000	8	0.264
			#DIV/0!		#DIV/0!		#DIV/0!		0.580

Figure 3: Absorbance and Activity Data for Experiment 60 Samples

Samples tested are listed by column. Each sample has two replicates, to ensure repeatability of data. Separate runs (rows) were performed on each sample as needed, to attain data within the desired range.

The numbers in the first row of each run are the absorbance reading for each sample replicate. The average for each replicate pair is below the second replicate (light blue). The dilution factor for the replicate pair is below the first replicate (gray). The final activity is calculated from the absorbance and dilution, and is located below the absorbance average (yellow).

	Sample	e61f1s8		e61f2s5		e61f2s6		e61f2s8		e61f3s5			e61f3s6		e61f3s8	
Run		Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2		Replicate 1	Replicate 2	Replicate 1	Replicate 2
1	Abs.	0.197	0.189	0.239	0.247	0.235	0.241	0.242	0.241							
	dilutn/avg	10	0.193	10	0.243	10	0.238	10	0.242		0.000			0.000		0.000
	Activity		0.307		0.378		0.371		0.376		#DIV/0!			#DIV/0!		#DIV/0!
	Abs.	0.129	0.126	0.185	0.188	0.191	0.191	0.182	0.191							
	dilutn/avg	20	0.128	20	0.187	20	0.191	20	0.187		0.000			0.000		0.000
	Activity		0.107		0.149		0.152		0.149		#DIV/0!			#DIV/0!		#DIV/0!
2	Abs.	0.217	0.212							0.268	0.269		0.265	0.265	0.262	0.266
	dilutn/avg	9	0.215		0.000		0.000		0.000	10	0.269		10	0.265	10	0.264
	Activity		0.375		#DIV/0!		#DIV/0!		#DIV/0!		0.414			0.409		0.408
	Abs.	0.219	0.223							0.213	0.207		0.215	0.212	0.215	0.216
	dilutn/avg	8	0.221		0.000		0.000		0.000	20	0.210		20	0.214	20	0.216
	Activity		0.433		#DIV/0!		#DIV/0!		#DIV/0!		0.165			0.168		0.169

Figure 4: Absorbance and Activity Data for Experiment 61 Samples

Samples tested are listed by column. Each sample has two replicates, to ensure repeatability of data. Separate runs (rows) were performed on each sample as needed, to attain data within the desired range.

The numbers in the first row of each run are the absorbance reading for each sample replicate. The average for each replicate pair is below the second replicate (light blue). The dilution factor for the replicate pair is below the first replicate (gray). The final activity is calculated from the absorbance and dilution, and is located below the absorbance average (yellow).

APPENDIX E

Rheology Data

- 1 – Solka-FlocTM slurry calculational data**
- 2 - Power Law Model Representation of Solka-FlocTM Slurry**
- 3 - Casson Model Representation of Solka-FlocTM Slurry**
- 4 – Broth and Solka-FlocTM slurry calculational data**
- 5 – Power Law Model Representation of T. reesei Broth and Solka-FlocTM**
- 6 - Casson Model Representation of T. reesei and Solka-FlocTM**
- 7 – Standards calculational data**
- 8 – Sucrose standards chart**
- 9 – Parameter calculation data**
- 10 – Experiment 62 calculational data**
- 11 – Experiment 62 Power Law Model Representation**
- 12 – Experiment 62 Casson Model Representation**
- 13 - Power Law Model Parameters with Cell and Substrate Concentrations**
- 14 - Casson Model Parameters with Cell and Substrate Concentrations**
- 15 - Solids Concentrations**

Weight (g)	Pure Water	Slurry 1%	Slurry 2%	Slurry 3%	Slurry 4%	Slurry 5%	Slurry 6%							
55	19	20.3	20.7	28	103.7									
60	18	18	20.3	26.7	78.3									
70	16	16.7	17	23.5	57.8									
100	13.7	13.3	14	18	33.3									
150	7.7	9	8	10.7	21	40								
200		7	6.7	7.3	17	29								
250				6	14	22	35.3							
300				5	11	17.7	28							
350							23.7							
400							20							
450							18							
500							16							
550														
600														
700														
800														
Shear Rates vs Shear Stress														
	Shear Rt	Shear Str	Shear Rt	Shear Str	Shear Rt	Shear Str	Shear Rt	Shear Str	Shear Rt	Shear Str	Shear Rt	Shear Str	Shear Rt	Shear Str
55	52.63	1536.15	49.26	1536.15	48.31	1536.15	35.71	1536.15	9.64	1536.15		1536.15		1536.15
60	55.56	1675.80	55.56	1675.80	49.26	1675.80	37.45	1675.80	12.77	1675.80		1675.80		1675.80
70	62.50	1955.10	59.88	1955.10	58.82	1955.10	42.55	1955.10	17.30	1955.10		1955.10		1955.10
100	72.99	2793.00	75.19	2793.00	71.43	2793.00	55.56	2793.00	30.03	2793.00		2793.00		2793.00
150	129.87	4189.50	111.11	4189.50	125.00	4189.50	93.46	4189.50	47.62	4189.50	25.00	4189.50		4189.50
200		5586.00	142.86	5586.00	149.25	5586.00	136.99	5586.00	58.82	5586.00	34.48	5586.00		5586.00
250		6982.50		6982.50		6982.50	166.67	6982.50	71.43	6982.50	45.45	6982.50	28.33	6982.50
300		8379.00		8379.00		8379.00	200.00	8379.00	90.91	8379.00	56.50	8379.00	35.71	8379.00
350		9775.50		9775.50		9775.50		9775.50		9775.50		9775.50	42.19	9775.50
400		11172.00		11172.00		11172.00		11172.00		11172.00		11172.00	50.00	11172.00
450		12568.50		12568.50		12568.50		12568.50		12568.50		12568.50	55.56	12568.50
500		13965.00		13965.00		13965.00		13965.00		13965.00		13965.00	62.50	13965.00
550		15361.50		15361.50		15361.50		15361.50		15361.50		15361.50		15361.50
600		16758.00		16758.00		16758.00		16758.00		16758.00		16758.00		16758.00
700		19551.00		19551.00		19551.00		19551.00		19551.00		19551.00		19551.00
800		22344.00		22344.00		22344.00		22344.00		22344.00		22344.00		22344.00
Log (shear rate/shear stress)														
			1.693	3.186	1.684	3.186	1.553	3.186	0.984	3.186				
			1.745	3.224	1.693	3.224	1.573	3.224	1.106	3.224				
			1.777	3.291	1.770	3.291	1.629	3.291	1.238	3.291				
			1.876	3.446	1.854	3.446	1.745	3.446	1.478	3.446				
			2.046	3.622	2.097	3.622	1.971	3.622	1.678	3.622	1.398	3.622		
			2.155	3.747	2.174	3.747	2.137	3.747	1.770	3.747	1.538	3.747		
							2.222	3.844	1.854	3.844	1.658	3.844	1.452	3.844
							2.301	3.923	1.959	3.923	1.752	3.923	1.553	3.923
													1.625	3.990
													1.699	4.048
												1.745	4.099	
												1.796	4.145	
sqrt (shear rate/shear stress)														
			7.02	39.19	6.95	39.19	5.98	39.19	3.11	39.19				
			7.45	40.94	7.02	40.94	6.12	40.94	3.57	40.94				
			7.74	44.22	7.67	44.22	6.52	44.22	4.16	44.22				
			8.67	52.85	8.45	52.85	7.45	52.85	5.48	52.85				
			10.54	64.73	11.18	64.73	9.67	64.73	6.90	64.73	5.00	64.73		
			11.95	74.74	12.22	74.74	11.70	74.74	7.67	74.74	5.87	74.74		
							12.91	83.56	8.45	83.56	6.74	83.56	5.32	83.56
							14.14	91.54	9.53	91.54	7.52	91.54	5.98	91.54
													6.50	98.87
													7.07	105.70
												7.45	112.11	
												7.91	118.17	

Figure 1: Viscosity Data for Pure Solka-Floc™ Slurry

Power Law Model Representation of Solka-Floc Slurry

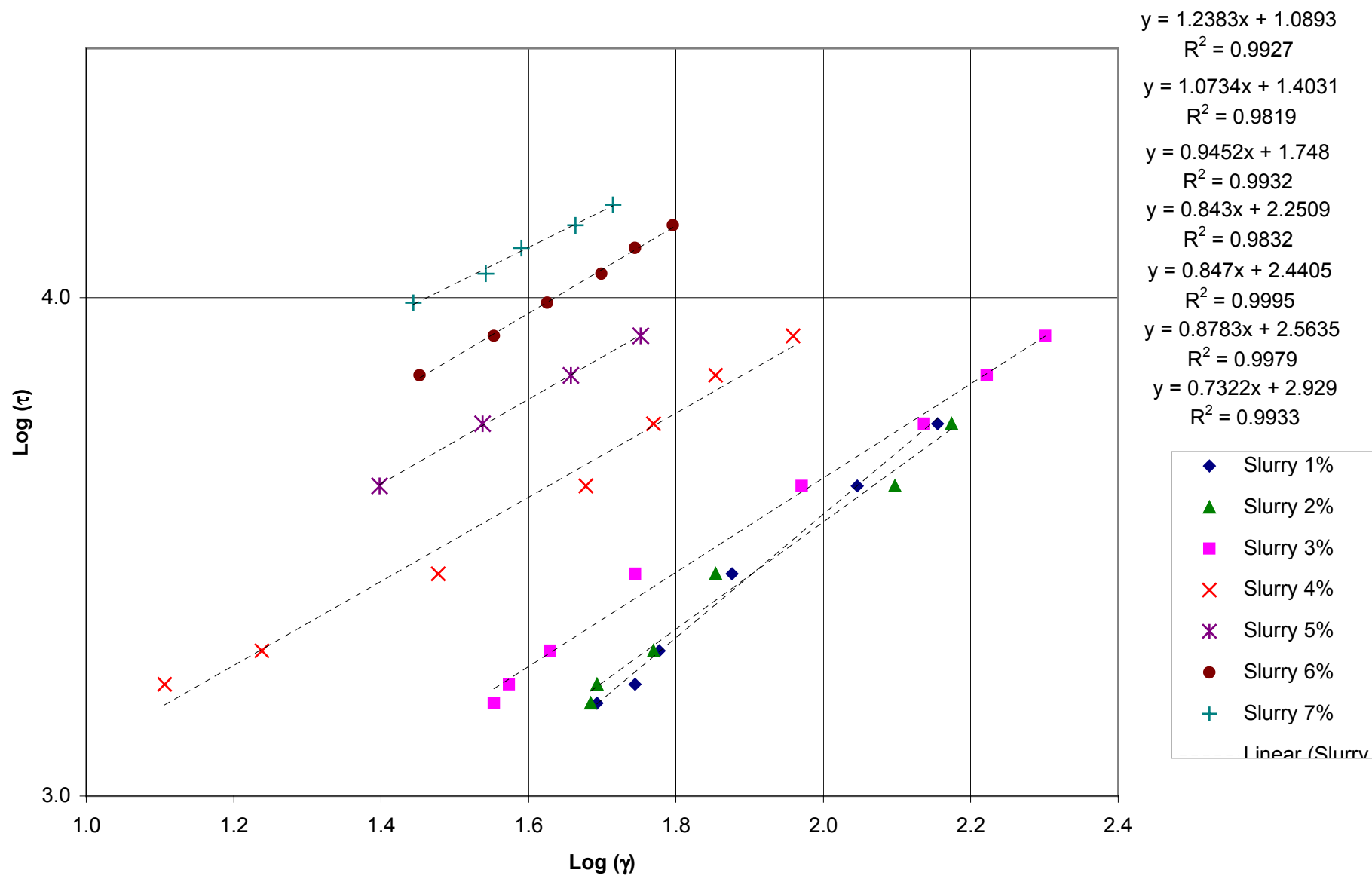


Figure 2: Power Law Model Representations for Pure Solka-Floc™ Slurry

Casson Model Representation of Solka-Floc Slurry

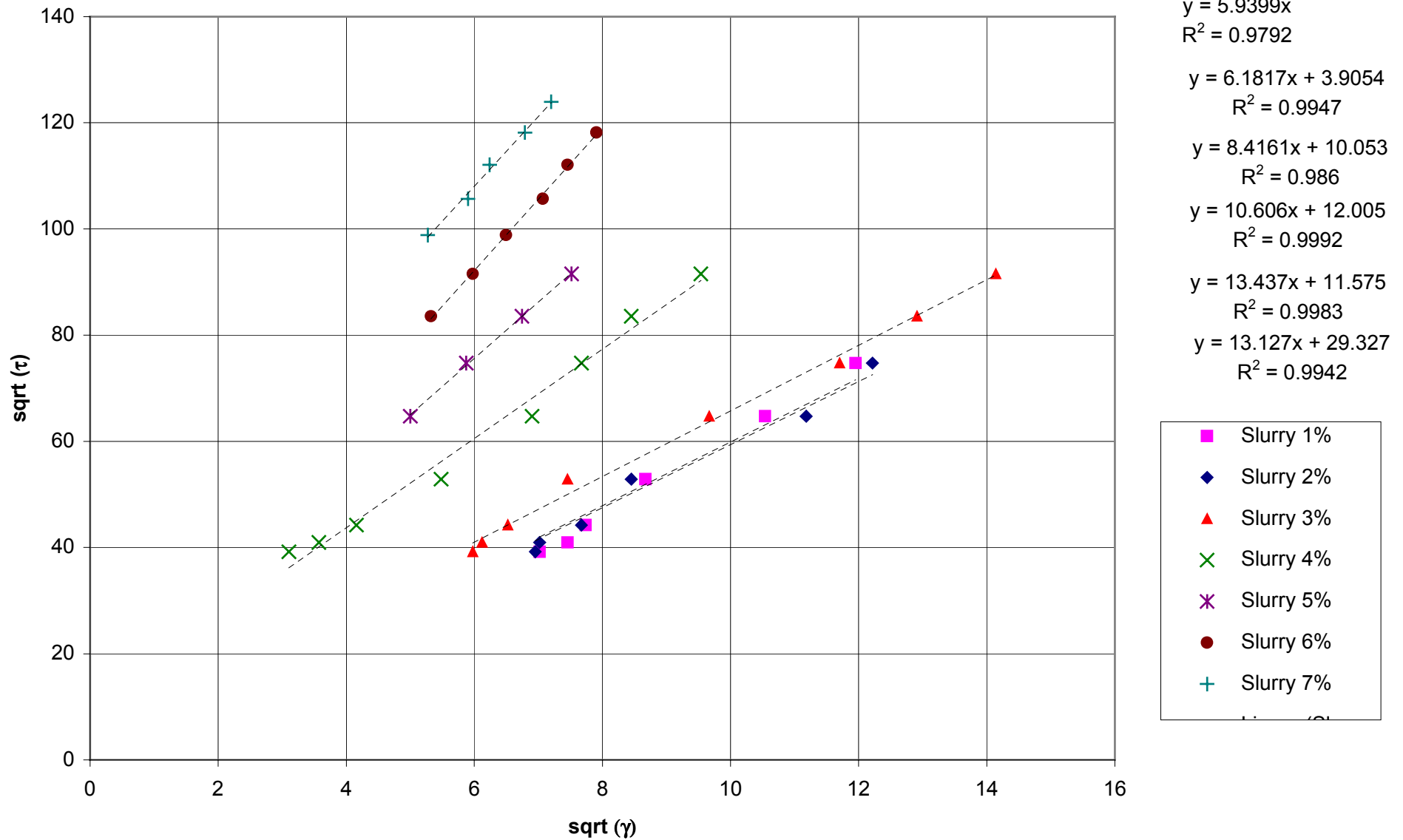
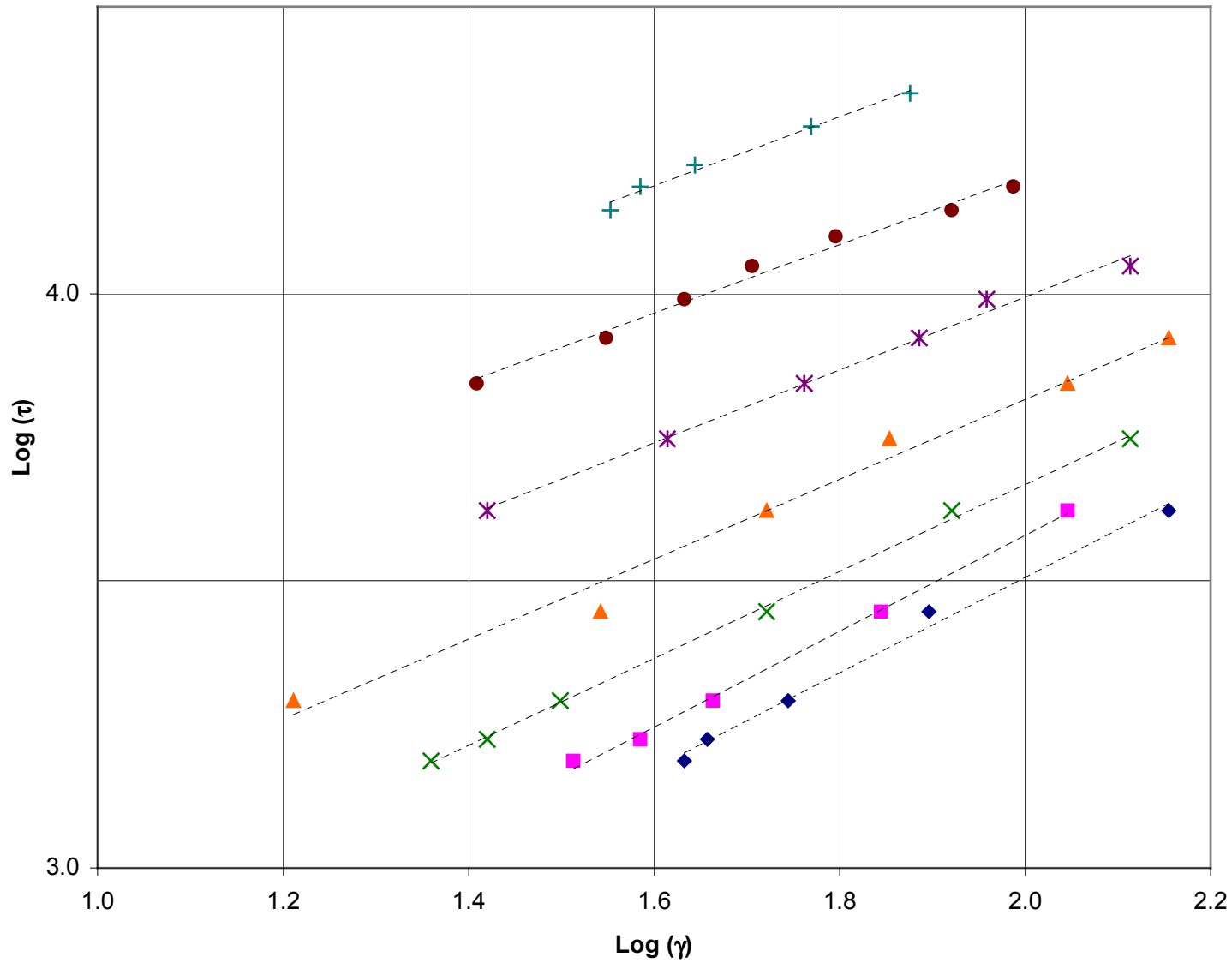


Figure 3: Casson Model Representations for Pure Solka-Floc™ Slurry

Weight (g)	T. reesei alone		T. reesei + 1% SF		T. reesei + 2% SF		T. reesei + 3% SF		T. reesei + 4% SF		T. reesei + 5% SF		T. reesei + 6% SF	
55	23.3		30.7		43.7									
60	22		26		38									
70	18		21.7		31.7		61.5							
100	12.7		14.3		19		28.7							
150	7		9		12		19		38					
200					7.7		14		24.3					
250							9		17.3		39			
300							7		13		28.3			
350									11		23.3			
400									7.7		19.7			
450											16			
500											12		28	
550											10.3		26	
600													22.7	
700													17	
800													13.3	
Shear Rate	Shear Rt	Shear Str	Shear Rt	Shear Str	Shear Rt	Shear Str	Shear Rt	Shear Str	Shear Rt	Shear Str	Shear Rt	Shear Str	Shear Rt	Shear Str
55	42.92	1536.15	32.57	1536.15	22.88	1536.15		1536.15		1536.15		1536.15		1536.15
60	45.45	1675.80	38.46	1675.80	26.32	1675.80		1675.80		1675.80		1675.80		1675.80
70	55.56	1955.10	46.08	1955.10	31.55	1955.10	16.26	1955.10		1955.10		1955.10		1955.10
100	78.74	2793.00	69.93	2793.00	52.63	2793.00	34.84	2793.00		2793.00		2793.00		2793.00
150	142.86	4189.50	111.11	4189.50	83.33	4189.50	52.63	4189.50	26.32	4189.50		4189.50		4189.50
200		5586.00		5586.00	129.87	5586.00	71.43	5586.00	41.15	5586.00		5586.00		5586.00
250		6982.50		6982.50		6982.50	111.11	6982.50	57.80	6982.50	25.64	6982.50		6982.50
300		8379.00		8379.00		8379.00	142.86	8379.00	76.92	8379.00	35.34	8379.00		8379.00
350		9775.50		9775.50		9775.50		9775.50	90.91	9775.50	42.92	9775.50		9775.50
400		11172.00		11172.00		11172.00		11172.00	129.87	11172.00	50.76	11172.00		11172.00
450		12568.50		12568.50		12568.50		12568.50		12568.50	62.50	12568.50		12568.50
500		13965.00		13965.00		13965.00		13965.00		13965.00	83.33	13965.00	35.71	13965.00
550		15361.50		15361.50		15361.50		15361.50		15361.50	97.09	15361.50	38.46	15361.50
600		16758.00		16758.00		16758.00		16758.00		16758.00		16758.00	44.05	16758.00
700		19551.00		19551.00		19551.00		19551.00		19551.00		19551.00	58.82	19551.00
800		22344.00		22344.00		22344.00		22344.00		22344.00		22344.00	75.19	22344.00
Log (shear rate)	Log (shear rate/shear stress)		Log (shear rate/shear stress)		Log (shear rate/shear stress)		Log (shear rate/shear stress)		Log (shear rate/shear stress)		Log (shear rate/shear stress)		Log (shear rate/shear stress)	
	1.633	3.186	1.513	3.186	1.360	3.186		3.291		3.622		3.844		4.145
	1.658	3.224	1.585	3.224	1.420	3.224		3.446		3.747		3.923		4.186
	1.745	3.291	1.664	3.291	1.499	3.291	1.211	3.291		3.923		3.990		4.224
	1.896	3.446	1.845	3.446	1.721	3.446	1.542	3.446		4.048		4.099		4.291
	2.155	3.622	2.046	3.622	1.921	3.622	1.721	3.622	1.420	3.622		4.145	1.553	4.145
					2.114	3.747	1.854	3.747	1.614	3.747		4.186	1.585	4.186
							2.046	3.844	1.762	3.844	1.409	3.844	1.644	4.224
							2.155	3.923	1.886	3.923	1.548	3.923	1.770	4.291
									1.959	3.990	1.633	3.990	1.876	4.349
									2.114	4.048	1.706	4.048		
											1.796	4.099		
											1.921	4.145		
											1.987	4.186		
sqrt (shear rate)	sqrt (shear rate/shear stress)		sqrt (shear rate/shear stress)		sqrt (shear rate/shear stress)		sqrt (shear rate/shear stress)		sqrt (shear rate/shear stress)		sqrt (shear rate/shear stress)		sqrt (shear rate/shear stress)	
	6.55	39.19	5.71	39.19	4.78	39.19		44.22		64.73		83.56	5.98	118.17
	6.74	40.94	6.20	40.94	5.13	40.94		40.94		74.74		91.54	6.20	123.94
	7.45	44.22	6.79	44.22	5.62	44.22	4.03	44.22		83.56		98.87	6.64	129.45
	8.87	52.85	8.36	52.85	7.25	52.85	5.90	52.85		91.54		105.70	7.67	139.82
	11.95	64.73	10.54	64.73	9.13	64.73	7.25	64.73	5.13	64.73		112.11	8.67	149.48
					11.40	74.74	8.45	74.74	6.42	74.74		118.17		
							10.54	83.56	7.60	83.56	5.06	83.56		
							11.95	91.54	8.77	91.54	5.94	91.54		
									9.53	98.87	6.55	98.87		
									11.40	105.70	7.12	105.70		
											7.91	112.11		
											9.13	118.17		
											9.85	123.94		

Figure 4: Viscosity Data for Solka-Floc™ Slurry in Fermentation Broth

Power Law Model Representation of T. reesei Broth and Solka-Floc



$$y = 0.8322x + 1.8417$$

$$R^2 = 0.9917$$

$$y = 0.8338x + 1.9113$$

$$R^2 = 0.9973$$

$$y = 0.7564x + 2.1545$$

$$R^2 = 0.9984$$

$$y = 0.6953x + 2.4254$$

$$R^2 = 0.9853$$

$$y = 0.6344x + 2.7254$$

$$R^2 = 0.9933$$

$$y = 0.5957x + 3.0126$$

$$R^2 = 0.9875$$

$$y = 0.6007x + 3.2268$$

$$R^2 = 0.9854$$

- ◆ T. reesei alone
- T. reesei + 1% SF
- × T. reesei + 2% SF
- ▲ T. reesei + 3% SF
- ✱ T. reesei + 4% SF
- T. reesei + 5% SF
- + T. reesei + 6% SF

Figure 5: Power Law Model Representation for Solka-Floc™ Slurry in Fermentation Broth

Casson Model Representation of T. reesei and Solka-Floc

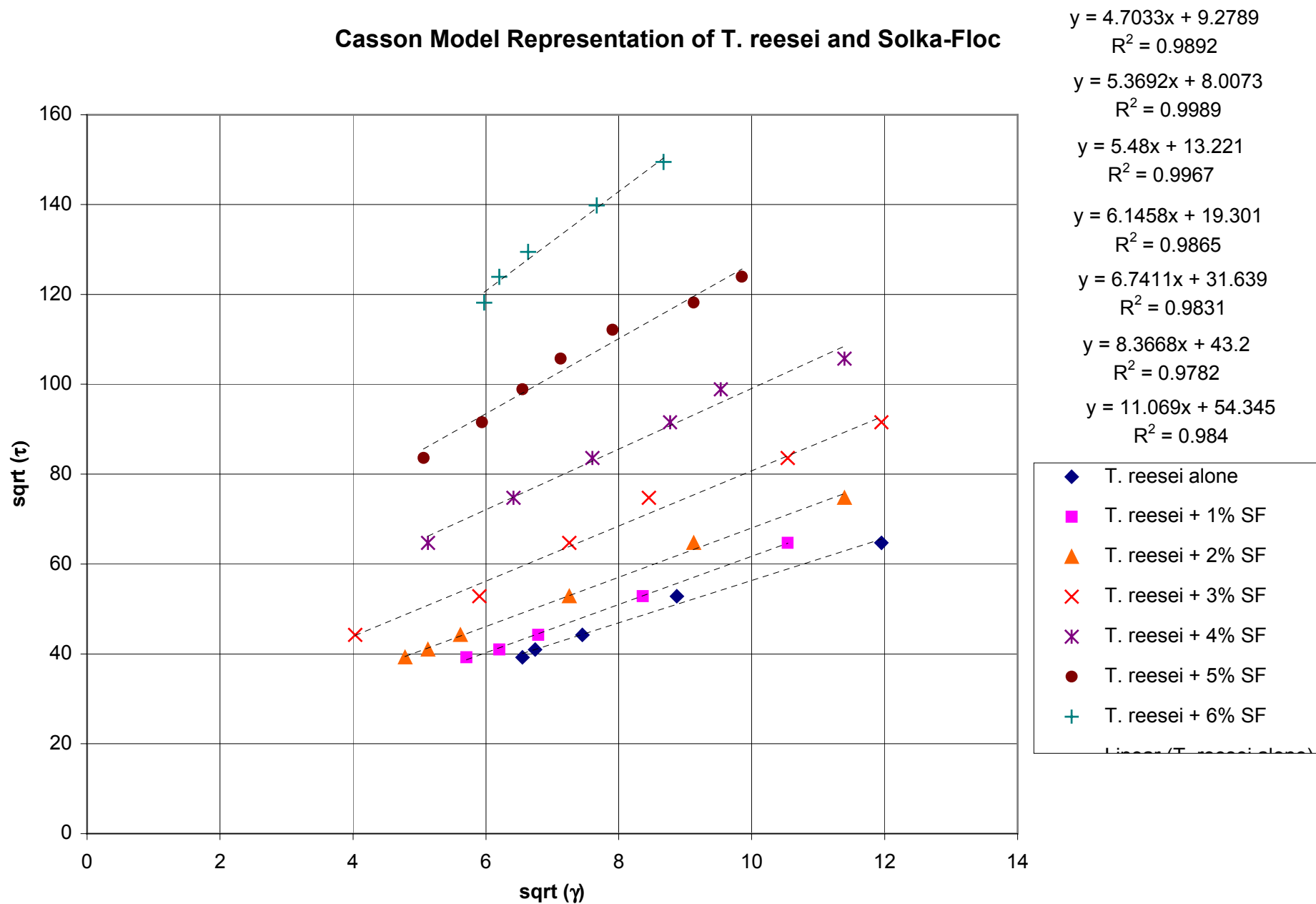


Figure 6: Casson Model Representation for Solka-Floc™ Slurry in Fermentation Broth

Sucrose Standards

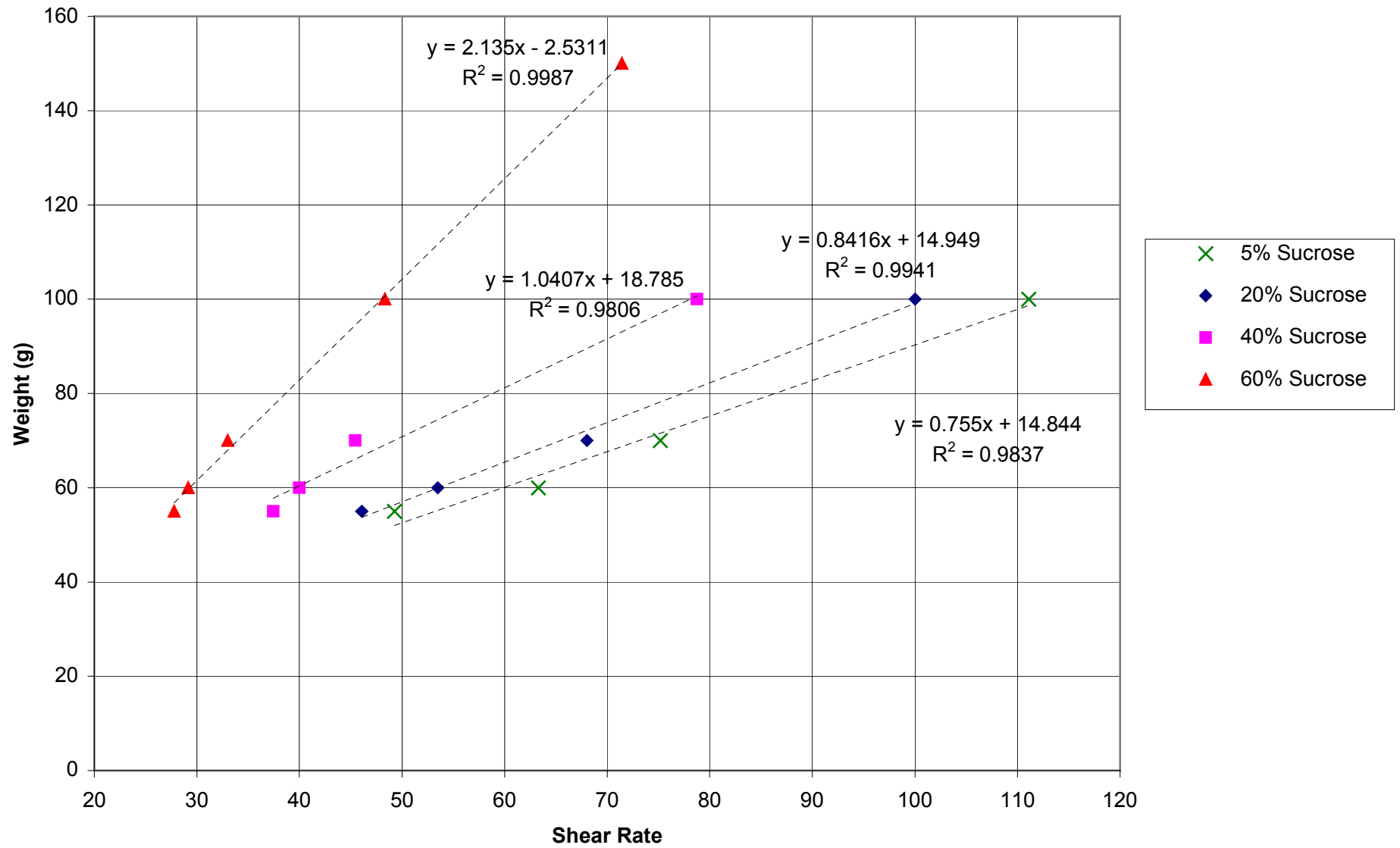


Figure 8: Sucrose Standards Curves

Slurry								T. reesei						
	Log			Casson					Log			Casson		
	n	log K	K	sqrt τ	τ	K			n	log K	K	sqrt τ	τ	K
1%	1.2383	1.0893	12.28	0	0.00	5.986		alone	0.8322	1.8417	69.45	9.2789	86.10	4.7033
2%	1.0734	1.4031	25.30	0	0.00	5.9399		+1%	0.8338	1.9113	81.53	8.0073	64.12	5.3692
3%	0.9452	1.748	55.98	3.9054	15.25	6.1817		+2%	0.7564	2.1545	142.72	13.221	174.79	5.48
4%	0.843	2.2509	178.20	10.053	101.06	8.4161		+3%	0.6953	2.4254	266.32	19.301	372.53	6.1458
5%	0.847	2.4405	275.74	12.005	144.12	10.606		+4%	0.6344	2.7254	531.37	31.639	1001.03	6.7411
6%	0.8783	2.5635	366.02	11.575	133.98	13.437		+5%	0.5957	3.0126	1029.44	43.2	1866.24	8.3668
7%	0.7322	2.929	849.18	29.327	860.07	13.127		+6%	0.6007	3.2268	1685.78	54.345	2953.38	11.069

Figure 9: Data Table of Unknowns for Pure Solka-Floc™ Slurry and Solka-Floc™ Slurry in Fermentation Broth for Both Power Law and Casson Models

Each row represents a different Solka-Floc™ concentration. The columns show the values determined for each unknown parameter for each model, and any conversions required.

* See Appendix A, Figures 2 & 3 for graphical representations of the above data.

Weight (g)	E62S1		E62S2		E62S3		E62S4		E62S5		E62S6		E62S7	
55			31.3		38.3		50.3		36.7		32.7		26.5	
60			30		33.3		41.7		32.7		29.7		23.3	
70	40.5		22.3		27		33.7		27.3		26		19	
100	31		13.3		17		21.3		18.3		16.7		13.3	
150	14.3		8.3		12		12		12		12			
200	8.7													
Shear Rates vs Shear Stress														
	Shear Rt	Shear Str	Shear Rt	Shear Str	Shear Rt	Shear Str	Shear Rt	Shear Str	Shear Rt	Shear Str	Shear Rt	Shear Str	Shear Rt	Shear Str
55		1536.15	31.95	1536.15	26.11	1536.15	19.88	1536.15	27.25	1536.15	30.58	1536.15	37.74	1536.15
60		1675.80	33.33	1675.80	30.03	1675.80	23.98	1675.80	30.58	1675.80	33.67	1675.80	42.92	1675.80
70	24.69	1955.10	44.84	1955.10	37.04	1955.10	29.67	1955.10	36.63	1955.10	38.46	1955.10	52.63	1955.10
100	32.26	2793.00	75.19	2793.00	58.82	2793.00	46.95	2793.00	54.64	2793.00	59.88	2793.00	75.19	2793.00
150	69.93	4189.50	120.48	4189.50	83.33	4189.50	83.33	4189.50	83.33	4189.50	83.33	4189.50		4189.50
200	114.94	5586.00		5586.00		5586.00		5586.00		5586.00		5586.00		5586.00
Log (shear rate/shear stress)														
		3.186	1.504	3.186	1.417	3.186	1.298	3.186	1.435	3.186	1.485	3.186	1.577	3.186
		3.224	1.523	3.224	1.478	3.224	1.380	3.224	1.485	3.224	1.527	3.224	1.633	3.224
	1.393	3.291	1.652	3.291	1.569	3.291	1.472	3.291	1.564	3.291	1.585	3.291	1.721	3.291
	1.509	3.446	1.876	3.446	1.770	3.446	1.672	3.446	1.738	3.446	1.777	3.446	1.876	3.446
	1.845	3.622	2.081	3.622	1.921	3.622	1.921	3.622	1.921	3.622	1.921	3.622		3.622
	2.060	3.747		3.747		3.747		3.747		3.747		3.747		3.747
sqrt (shear rate/shear stress)														
		39.19	5.65	39.19	5.11	39.19	4.46	39.19	5.22	39.19	5.53	39.19	6.14	39.19
		40.94	5.77	40.94	5.48	40.94	4.90	40.94	5.53	40.94	5.80	40.94	6.55	40.94
	4.97	44.22	6.70	44.22	6.09	44.22	5.45	44.22	6.05	44.22	6.20	44.22	7.25	44.22
	5.68	52.85	8.67	52.85	7.67	52.85	6.85	52.85	7.39	52.85	7.74	52.85	8.67	52.85
	8.36	64.73	10.98	64.73	9.13	64.73	9.13	64.73	9.13	64.73	9.13	64.73		64.73
	10.72	74.74		74.74		74.74		74.74		74.74		74.74		74.74

Figure 10: Viscosity Data for Experiment 62 Fermentation Samples

Power Law Model Representation of Fermentation Broth for Experiment 62

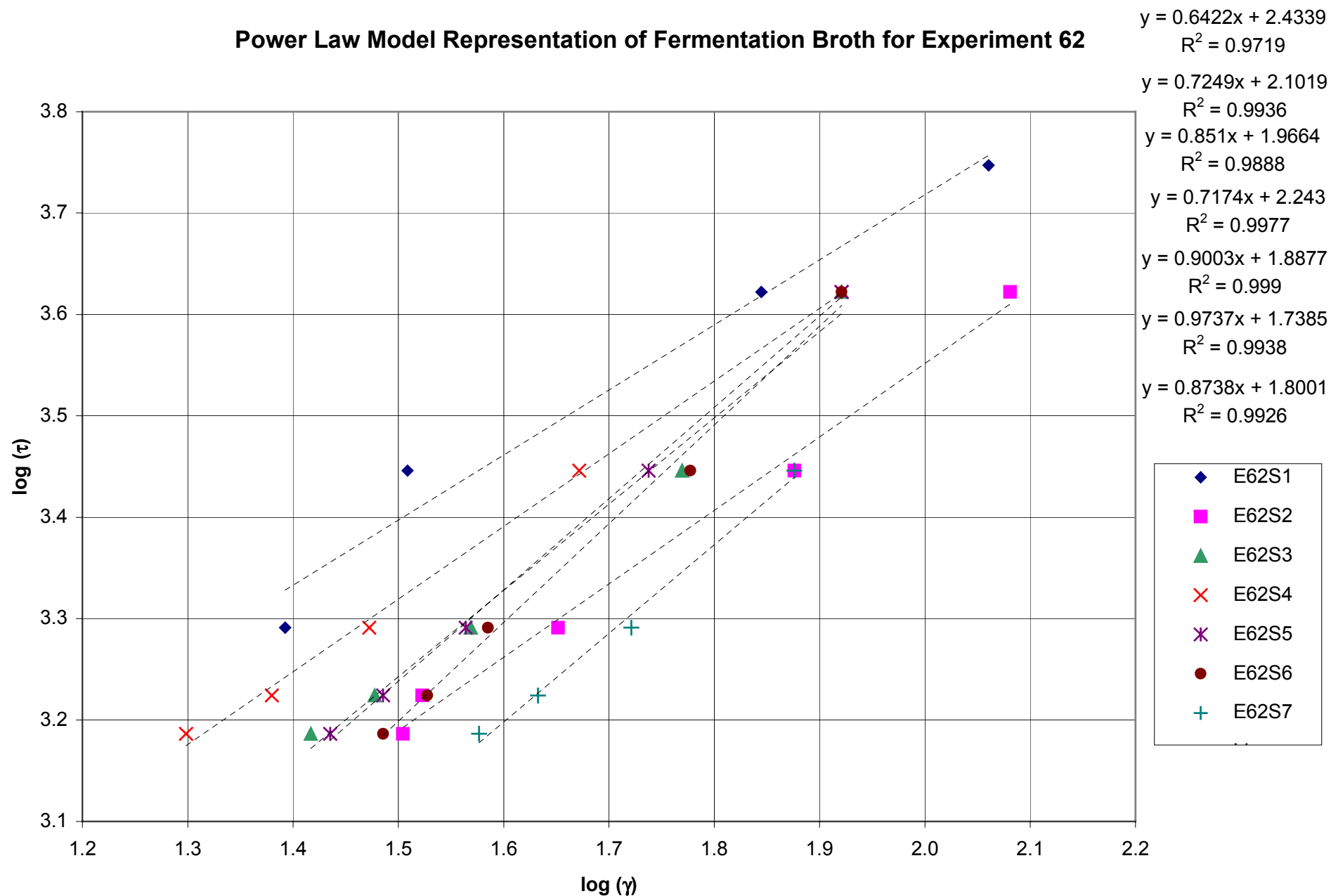


Figure 11: Power Law Model Representation of the Experiment 62 Fermentation Broth

Casson Model Representation of Fermentation Broth for Experiment 62

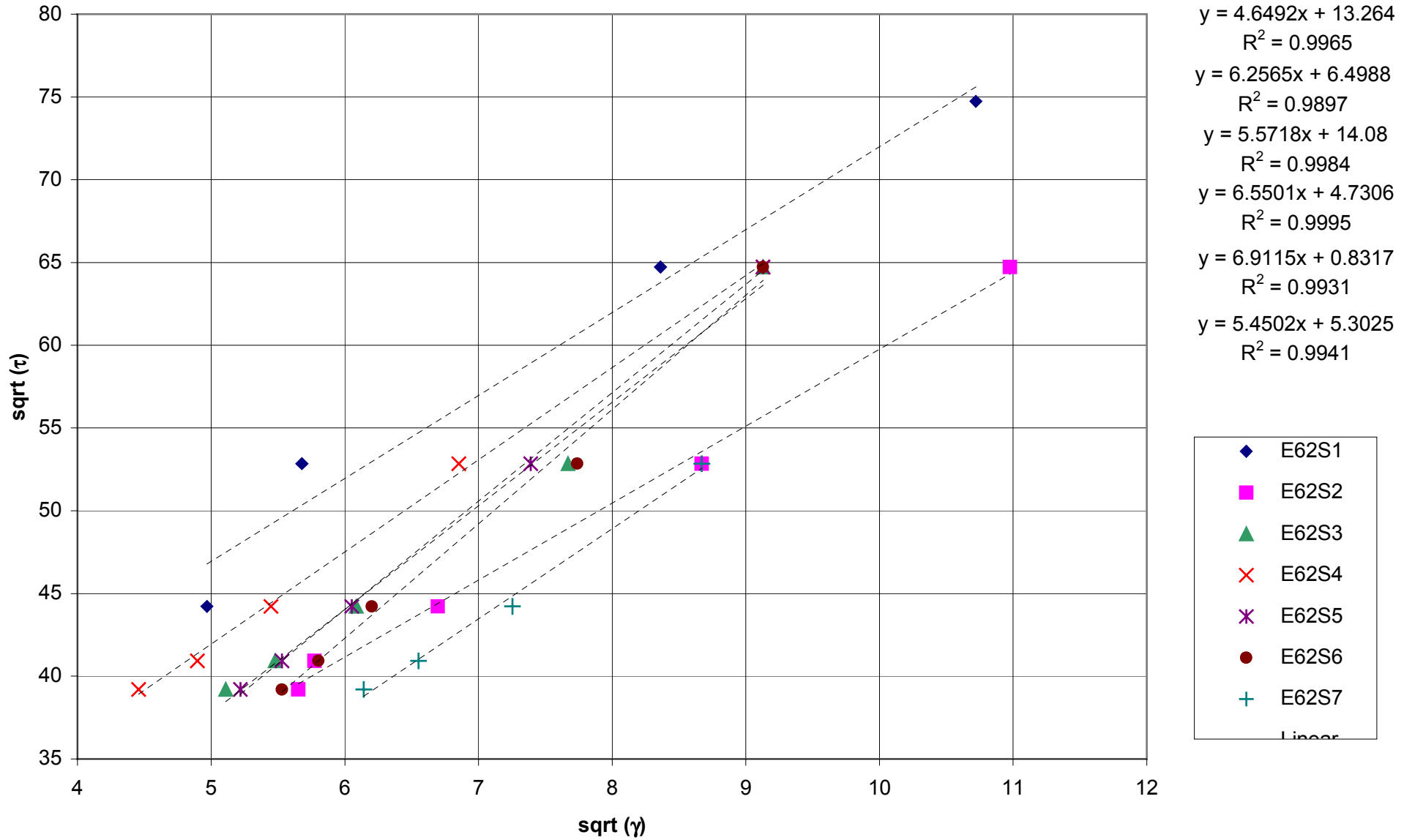


Figure 12: Casson Model Representation for the Experiment 62 Fermentation Broth

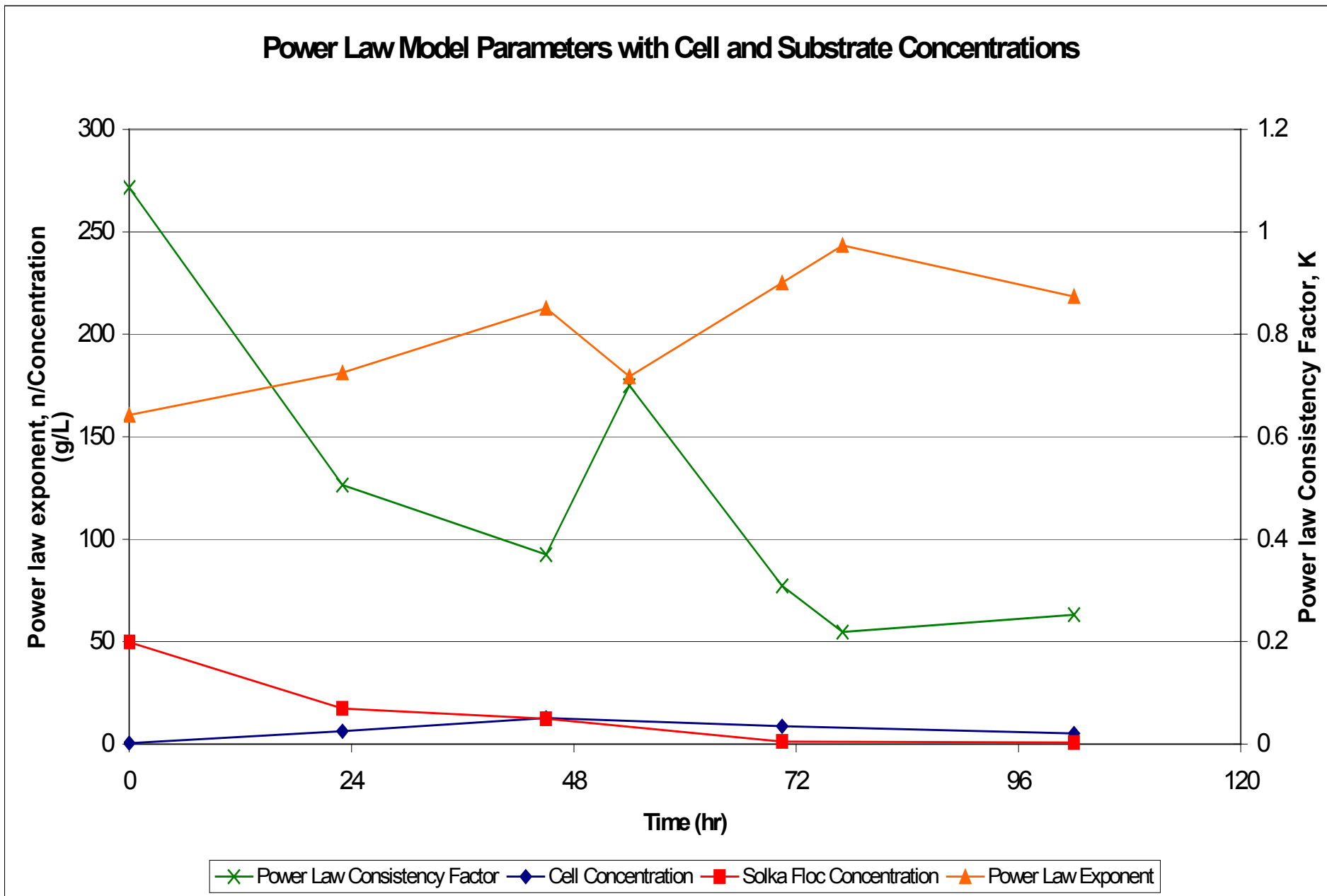


Figure 13: Graph of Power Law Model Parameters with Overall Solids Concentration

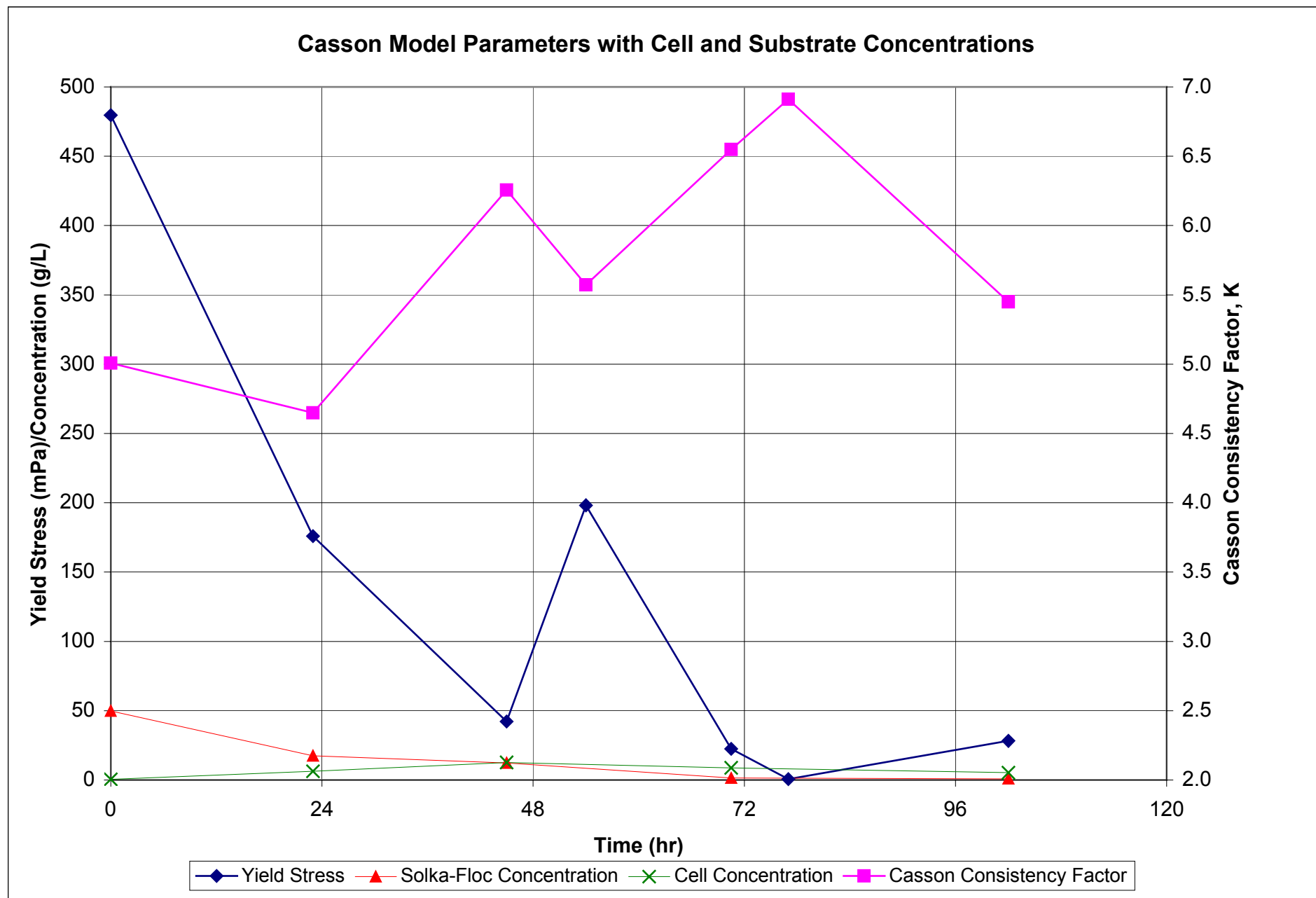


Figure 14: Graph of Casson Model Parameters with Overall Solids Concentration

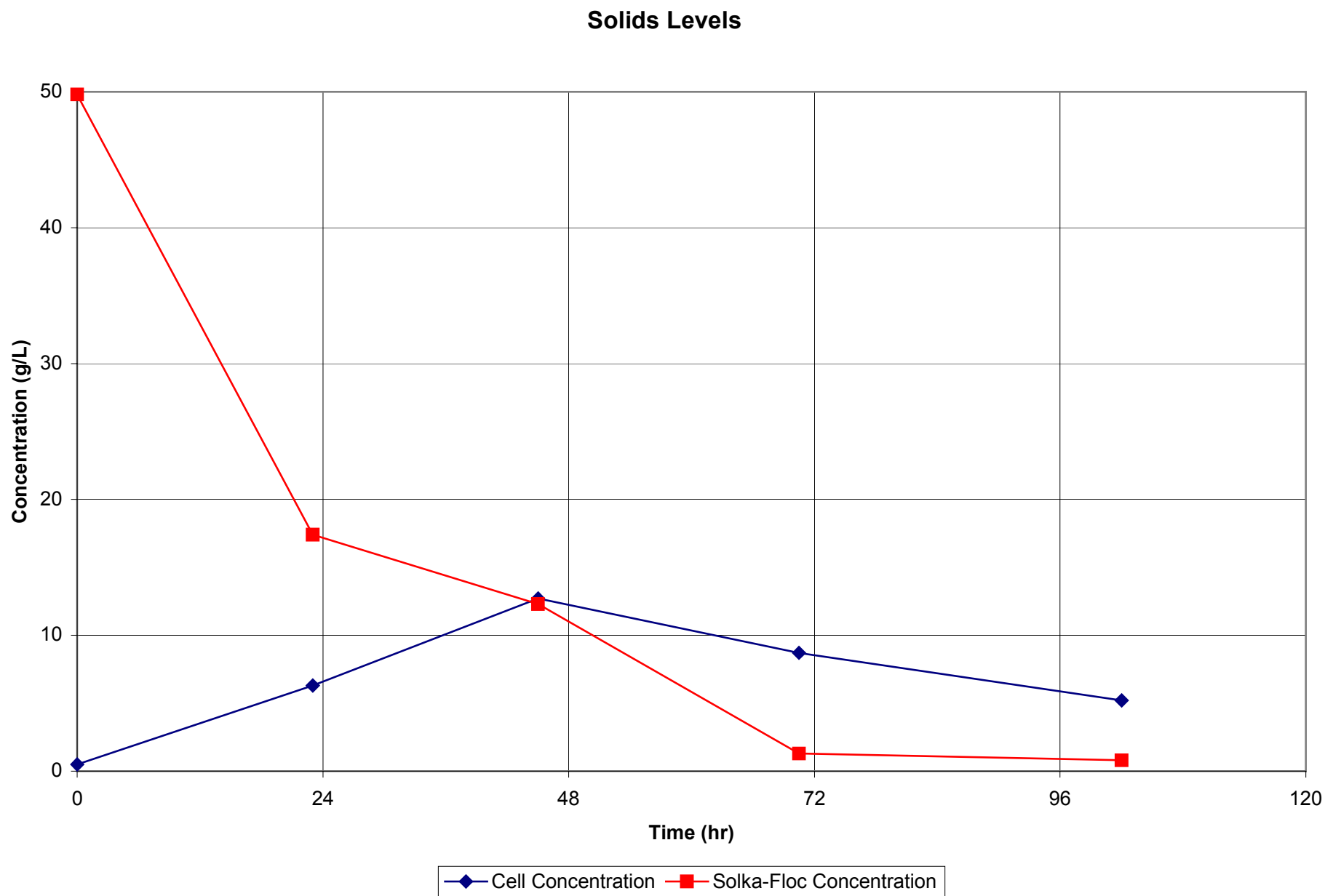


Figure 15: Change in Total Solids Concentration over Fermentation Lifetime

APPENDIX F

Fed-batch Addition System

- 1 – Operational Testing Data**
- 2 – Sample Moisture Content and Controller Setup**
- 3 – Representation of Average Flow for Each Substrate**
- 4 – Stirrer Modification**

Cycle #	SF (g)	cycle diff	Sawdust		Sawdust Run #2		Purge=10psi Wet Stover		Purge=20psi Wet Stover		4 month Dry Stover		1 week Dry Stover	
1	0.07	0.07	0.51	0.51	0.66	0.66	1.36	1.36	2.03	2.03	2.95	2.95	2.34	2.34
2	0.12	0.05	1.16	0.65	1.19	0.53	2.42	1.06	2.59	0.56	5.75	2.80	4.59	2.25
3	0.25	0.13	1.64	0.48	1.96	0.77	3.24	0.82	3.38	0.79	8.47	2.72	6.94	2.35
4	0.47	0.22	2.08	0.44	2.71	0.75	4.11	0.87	4.10	0.72	10.66	2.19	9.16	2.22
5	0.60	0.13	2.81	0.73	3.39	0.68	5.11	1.00	5.00	0.90	10.88	0.22	11.69	2.53
6	0.71	0.11	3.41	0.60	3.99	0.60	5.72	0.61	5.70	0.70	10.90	0.02	14.03	2.34
7	0.82	0.11	3.85	0.44	4.65	0.66	7.03	1.31	6.84	1.14			16.18	2.15
8	1.06	0.24	4.39	0.54	5.24	0.59	8.13	1.10	8.17	1.33	Avg	1.82	18.33	2.15
9	1.26	0.20	5.01	0.62	5.75	0.51	9.44	1.31	10.07	1.90	Std Dev	1.340	18.78	0.45
10	1.38	0.12	5.55	0.54	6.27	0.52	9.93	0.49	11.09	1.02			19.84	1.06
11	1.50	0.12	6.37	0.82	7.17	0.90	10.22	0.29	12.13	1.04	Values when end effects removed Avg 2.67 Std Dev 0.331 (at cycle #4)		22.10	2.26
12	1.59	0.09	6.94	0.57	7.84	0.67	10.74	0.52	13.17	1.04			23.90	1.80
13	1.64	0.05	7.47	0.53	8.51	0.67	11.40	0.66	14.50	1.33			24.77	0.87
14	1.79	0.15	7.74	0.27	9.14	0.63	11.98	0.58	15.04	0.54			24.84	0.07
15	2.04	0.25	8.21	0.47	9.70	0.56	12.17	0.19	15.68	0.64			24.89	0.05
16	2.16	0.12	8.85	0.64	10.06	0.36	12.51	0.34	16.26	0.58	Avg 1.66 Std Dev 0.896			
17	2.27	0.11	9.38	0.53	10.73	0.67			16.65	0.39				
18	2.40	0.13	9.90	0.52	11.49	0.76	Avg	0.78	17.54	0.89				
19	2.56	0.16	11.16	1.26	12.66	1.17	Std Dev	0.379	18.02	0.48	Values when end effects removed Avg 1.91 Std Dev 0.667 (at cycle #13)			
20	2.66	0.10	11.85	0.69	13.35	0.69			18.60	0.58				
21	3.00	0.34	12.44	0.59	14.88	1.53			18.92	0.32				
22	3.17	0.17	13.06	0.62	15.97	1.09			20.68	1.76				
23	3.31	0.14	13.64	0.58	16.33	0.36			21.25	0.57				
24	3.36	0.05	14.37	0.73	16.69	0.36			21.77	0.52				
25	3.52	0.16	15.01	0.64	17.35	0.66			23.18	1.41				
26	3.72	0.20	16.05	1.04	17.80	0.45			25.92	2.74				
27	3.89	0.17	17.46	1.41	18.19	0.39			27.12	1.20				
28	4.07	0.18	18.57	1.11	18.59	0.40			28.09	0.97				
29	4.31	0.24	19.13	0.56	19.02	0.43			28.98	0.89				
30	4.58	0.27	20.63	1.50	19.28	0.26			29.36	0.38				
31	4.72	0.14	21.27	0.64	19.55	0.27			29.65	0.29				
32	4.80	0.08	23.31	2.04	19.78	0.23			29.95	0.30				
33	5.00	0.20	23.70	0.39	20.02	0.24			30.21	0.26				
34	5.11	0.11	24.15	0.45	20.26	0.24			30.48	0.27				
35	5.30	0.19	24.41	0.26	20.68	0.42			30.64	0.16				
36	5.44	0.14	25.51	1.10	20.99	0.31			30.88	0.24				
37	5.59	0.15	26.38	0.87	21.53	0.54			30.95	0.07				
38	5.71	0.12	27.00	0.62	22.08	0.55			30.98	0.03				
39	5.81	0.10	27.59	0.59	22.50	0.42			31.07	0.09				
40	5.92	0.11	28.02	0.43	23.06	0.56			31.23	0.16				
41	6.25	0.33	28.53	0.51	23.77	0.71			31.65	0.42				
42	6.38	0.13	29.04	0.51	24.26	0.49			32.30	0.65				
43	6.70	0.32	29.94	0.90	25.05	0.79			32.49	0.19				
44	6.85	0.15	31.84	1.90	25.58	0.53			32.64	0.15				
45	6.96	0.11	32.43	0.59	26.57	0.99			33.28	0.64				
46	7.05	0.09	32.81	0.38	27.27	0.70			33.54	0.26				
47	7.16	0.11	34.30	1.49	28.01	0.74			33.92	0.38				
48	7.34	0.18	37.03	2.73	28.66	0.65			33.93	0.01				
49	7.46	0.12	39.29	2.26	29.27	0.61			34.02	0.09				
50	7.58	0.12	40.20	0.91	29.77	0.50			34.20	0.18				
Average cycle value		0.15	0.80		0.60				0.68					
Standard deviation		0.068	0.520		0.248				0.572					
												Values when end effects removed (at cycle #32)		
												0.94		
												0.563		
												Values when end effects removed (at cycle #29)		
												1.00		
												0.554		

Table 1: Fed-batch System Operational Testing Data

Moisture Content

Material	Density	Moisture (% solids)
Solka-Floc	0.057	100.00
Sawdust	0.267	52.97
Wet Stover	0.333	35.98
Dry Stover (1 week)	0.327	75.94
Dry Stover (4 months)	0.279	83.81

Table 2a: Moisture Content for Different Substrates

Controller Program

	1	2	3	4
15				X
0.1	X			X
10		X		X
2				X
3			X	X

Table 2b: Controller Operation Program

Column numbers represent the controller outputs

- 1: Upper Purge Valve
- 2: Pocketball Valve Actuator
- 3: Lower Purge Valve
- 4: Stirrer Motor

Time for operation of each step is down the first column, in seconds. An 'X' in the box represents power to that component during that step.

For purge valves, power applied indicates valve open.

For the actuator, power applied indicates valve in up position. Power off is down.

All valves fail to their power off positions.

Average Flow per Cycle for Tested Substrates

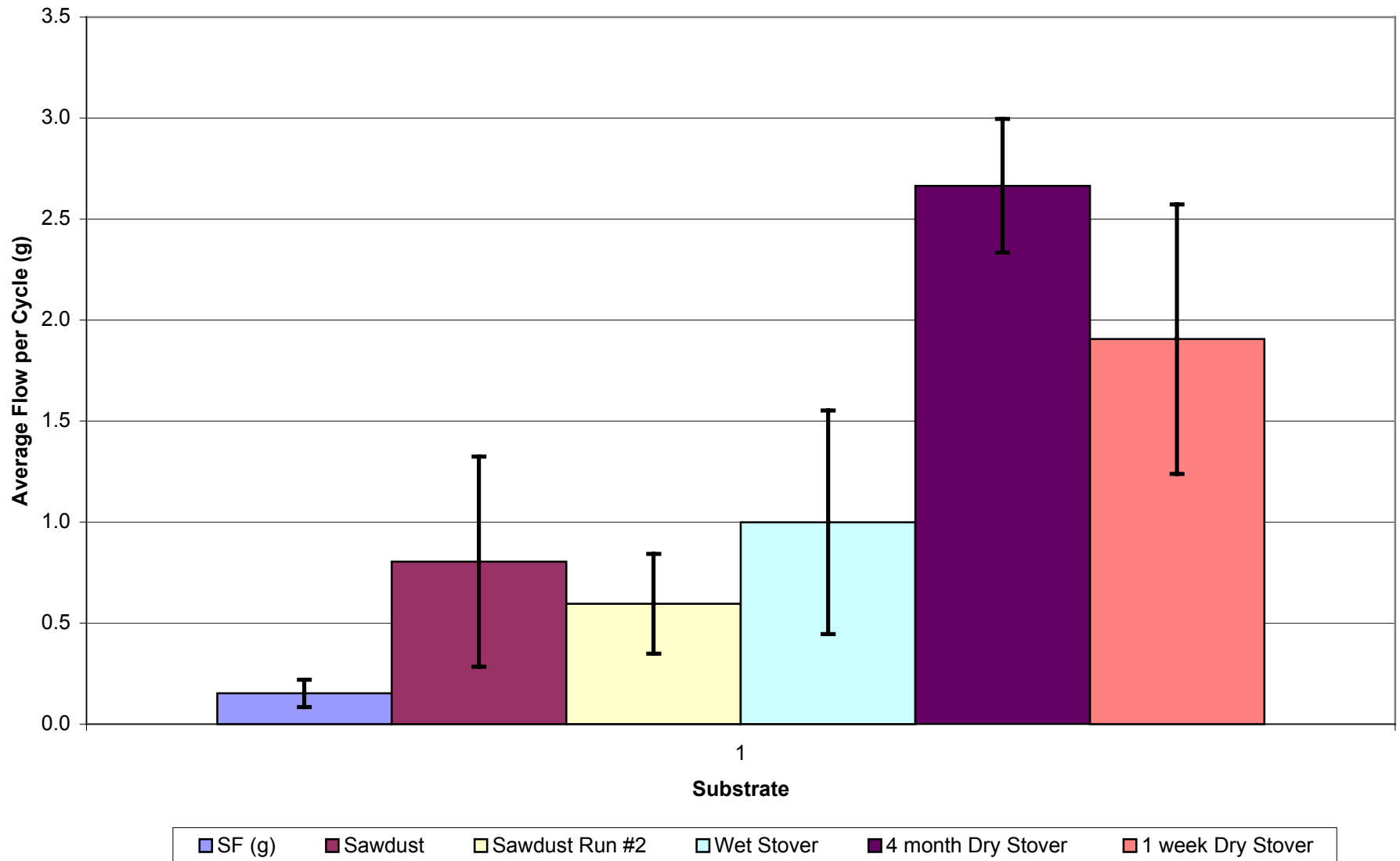


Figure 1: Representation of Average Flow for Each Substrate



Figure 2: Picture of Stirrer Modification to Hopper Lid